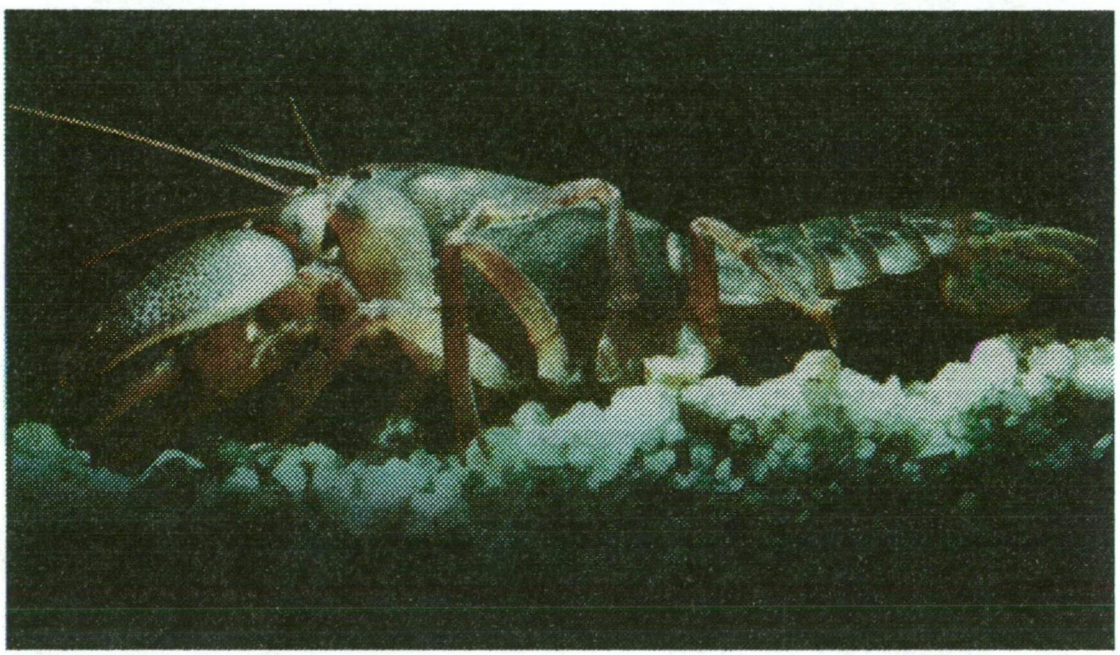
Systematics and Phylogeny of the Tasmanian freshwater crayfish genus *Parastacoides* (Decapoda: Parastacidae)



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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, the University of Tasmania (December, 2001)

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Brita Hansen

Date

20.11.2001

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Thesis Abstract

The endemic Tasmanian freshwater crayfish genus *Parastacoides* is confined to the western parts of the State. The most recent review of the genus recognised only one species, consisting of three sub-species. Since that review, extensive collection in remote areas of Tasmania had been undertaken and the diversity revealed by these collections suggested the need for a review. This thesis presents a complete review of the systematics of the genus, investigating morphological, molecular and biogeographical aspects of the taxon.

Two genera consisting of fourteen species are now recognised; suggested nomenclature, full descriptions, illustrations, distribution maps and keys are provided. Three species comprise the new genus *Spinastacoides* and 11 species comprise the new genus *Ombrastacoides*. The main diagnostic feature separating the two genera is the presence/absence of a terminal mesial spine on the uropod exopod. All species were found to be highly conservative morphologically, with few useful diagnostic characters. Paradoxically, a large degree of within-species plasticity was noted. Despite the high degree of morphological conservatism displayed, morphometric analyses confirmed that there was significant variation in shape between genera and species groups.

Distributions of genera and species were mapped and discussed, and possible influences determining the distribution are discussed. Distinctive differences were noted in the distributional patterns of the two genera. The genus *Spinastacoides* occupies the south-western region, with each species having similar distributions of similar area. The genus *Ombrastacoides* occurs throughout the western half of the state, however it is absent from the much of the region occupied by *Spinastacoides* species. *Ombrastacoides* species have widely differing ranges, with some species having extremely restricted distributions. The main factor limiting the distribution of the two genera to the western regions of Tasmania appears to be a combination of rainfall and evaporation rate; the rate must be sufficient to retain a degree of burrow moisture through the dryer summer months.

Molecular studies, involving allozyme electrophoresis, COI mtDNA and 16S mtDNA analyses, suggest that genetic distances between species are very high, and that the speciation events are ancient, occurring well before the Pleistocene glaciations, most probably during the Miocene. A study into the ecological niches occupied by the different species suggested that most species were generalists, able to exploit a wide variety of vegetation, substrate, temperature and altitude variables. Adaptive radiation could therefore be eliminated as a major determinant for the speciation and distribution of the species. A combination of ecological (the increasing aridity of the Australian climate, the vegetation turnover from C3 to C4 plants, the decrease in atmospheric CO_2 levels)and vicariant events (glaciations, volcanic activity, the Australian mainland and Tasmania becoming separated by the formation of Bass Strait) occurring during the Miocene are suggested as causes of speciation.

While some closely related sister-taxa clades are geographically based, overall the relationships between the phylogeny of these species and their geographic distributions are not straight forward, and some possible explanations are given. The sister-group relationship with South American and New Zealand taxa suggest the origin of the ancestral taxon during the Cretaceous, when these land masses were still connected. The origin was also most likely somewhere near the vicinity of the extant taxa.

Acknowledgments

There are many people to whom I owe thanks, and without whom this thesis would not have been possible.

Firstly, thanks must first go to my supervisor, Dr Alastair Richardson, whose enthusiasm for freshwater crayfish inspired me to appreciate the little creatures, and want to know more about them. His encouragement, guidance and support throughout the course of this study, his patience and willingness to discuss problems, and his comments on the drafts of this thesis, have ensured the whole process has been as painless as possible.

I would also like to thank Adam Smolenski, my technical supervisor, whose infinite patience and advice have made the molecular work possible. Thanks are also due to Adam for the many helpful comments on the drafts of the molecular section of this thesis.

Special thanks go to a number of members of staff at the School of Zoology: I thank Leon Barmuta, for invaluable advice with statistics, Kit Williams for saving me whenever computer problems arose, I would also like to thank Sherrin Bowden, Wayne Kelly and Barry Rumbold for their friendship and assistance during my time at Zoology.

I wish to thank the co-authors of the papers for their assistance, and for the helpful comments on the drafts. Thanks are also due to the Parks and Wildlife service for providing funds in the form of a Tasmanian Wilderness World Heritage Area Fauna Research Grant to assist in this study.

To all the people who have collected crayfish over the years, heartfelt thanks. Without the extensive collection at the School of Zoology, it would not be possible to collect enough material during the course of a PhD.

Thanks to all the other Postgrads (especially those who shared my office over the course of the study, Kerrie Swadling, Ashley Edwards, Beate Sterneberg and Heather Hesterman – wonderful people one and all) for interaction and help over the years.

And last, but by no means last, to my dear husband, Frank Halley, without whose support this entire project would most certainly not have been possible ..... I will never forget the support you have provided. Thank you.

Title: Systematics and Phylogeny of the Tasmanian freshwater crayfish genus *Parastacoides* (Decapoda: Parastacidae)

Section 3 – Biogeography and Evolutionary History

1. General Introduction

Introduction and Background

Freshwater crayfish are found on every continent except Antarctica, Africa and the Indian subcontinent (Hobbs 1988) (see Figure 1.1 for distribution details). All freshwater crayfish belong to the Infraorder Astacidea. Molecular (Crandall *et al.* 2000b) and morphological (Scholz 1999) studies have established freshwater crayfish as a monophyletic group. Morphological characters suggest freshwater crayfish are closely related to mud shrimps (Thalassinida) and not to clawed lobsters (Scholtz 1999), whereas molecular data suggests a closer affinity to the clawed lobsters (Crandall *et al.* 2000b). The Northern Hemisphere crayfish superfamily Astacoidea contains two families, the Cambaridae and Astacidae, but there is doubt concerning the monophyly of Cambaridae, since the genus *Cambaroides* has been associated with the Astacidae (Crandall *et al.* 2000b). In the present classification Cambarids are found in eastern North America and western Asia, while the Astacidae occur in western North America and Europe. All Southern Hemisphere freshwater crayfish belong to the superfamily Parastacoidea, consisting of one monophyletic family, the Parastacidae. The monophyly of Parastacoidea is not in doubt. Parastacids occur in South America, Madagascar, New Zealand, New Guinea and Australia.

With molecular and morphological data establishing freshwater crayfish as a monophyletic group, and the geographical distribution of the superfamilies, it is possible to suggest hypotheses regarding the timing of major events in crayfish evolution. Crandall *et al.* (2000b) suggest that, on the basis of this evidence, crayfish must have originated in Pangaea by the Triassic (185-225 million years ago). Fossil evidence also supports the existence of the stem species of all freshwater crayfish before the break-up of the Pangaean supercontinent (Babcock *et al.* 1998), and a Gondwanan origin for the parastacids. The separation of Pangaea into Laurasia and Gondwana, about 185 million years ago, allowed the development of the two superfamilies.

The timing of invasion into freshwater habitats and the number of invasions are still subject to some discussion (Scholtz 1999). The absence of freshwater crayfish from

the Gondwanan regions of Africa and the Indian sub-continent are yet to be satisfactorily explained. There are two possible explanations: 1) they were present at some time in the past, and have since disappeared or 2) they have never inhabited these regions. Hobbs (1988) raises the theory that Parastacidae were once more widespread than the current distribution, and that the advent of freshwater crabs may have eliminated the freshwater crayfish from Africa and the Indian subcontinent. Alternatively, the hypothesised presence of a link between Indo-Madagascar and Antarctica across the Kerguelen Plateau, persisting later than the separation between South America and Africa, is used by Samson *et al.* (1998) to explain the presence of predatory dinosaurs, the Abelisauridae, in Madagascar and not in Africa. This link would have allowed Late Cretaceous biota in South America and Indo-Madagascar to exhibit greater affinity than that shown between South American and African biota. While this theory does not explain the absence of freshwater crayfish from the Indian subcontinent, it does explain the presence of freshwater crayfish in Madagascar and their absence from Africa. There is at least one example of a plant family that is absent from Africa, and has an Australian, south-east Asian, Madagascan and Mascarene distribution: the Casuarinas (White 1993). So whilst a Gondwanan distribution excluding Africa is unusual, it is by no means unique.

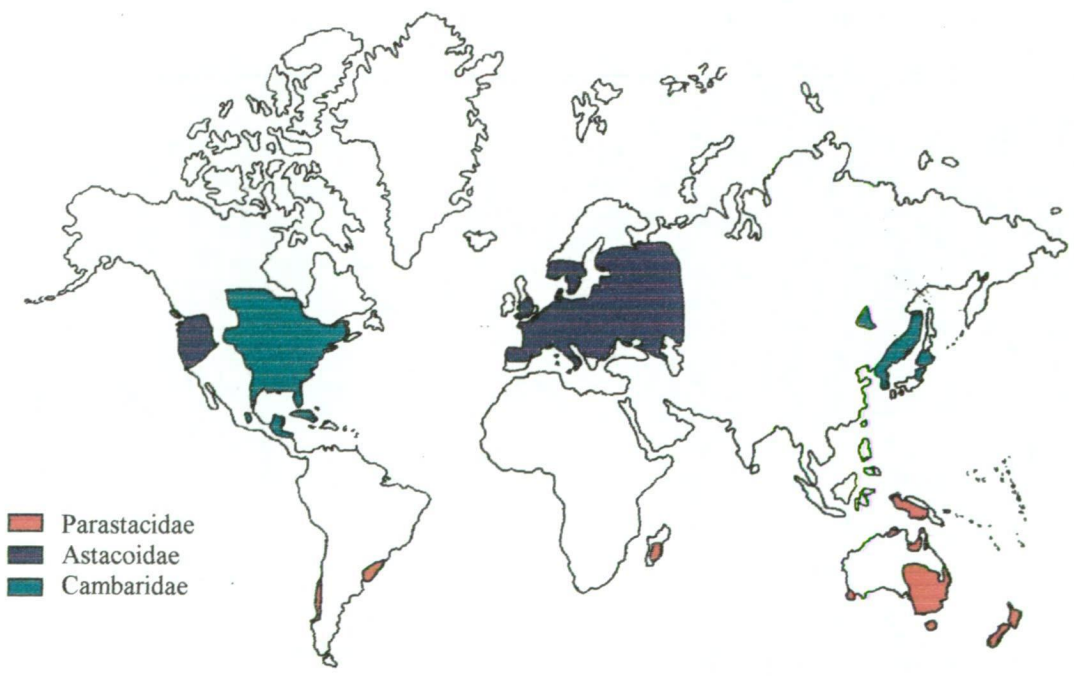


Figure 1.1 Geographical distribution of the freshwater crayfish families.

The family Cambaridae consists of 10 genera (Hobbs 1974) with over 350 species (Crandall *et al.* 2000b), and the family Astacidae has three genera consisting of approximately 12 species (Hobbs 1974). The family Parastacidae has currently 14 genera consisting of around 180 species (Crandall *et al.* 2000b). Of the 13 genera in the family Parastacidae, four are found in Tasmania: *Parastacoides* and *Astacopsis*, both of which are endemic to the State, and *Engaeus* and *Geocharax*, which are endemic to south-eastern Australia.

Fossil evidence, usually very important in the construction of phylogenies, is entirely lacking for *Parastacoides* species. Indeed, few known parastacid fossils exist: a Miocene *Paranephrops* specimen (Feldmann and Pole 1994), and a partial fossil believed to be related to the modern *Euastacus* genus (Sokal 1987).

The question of the monophyly of *Parastacoides* and the phylogeny of its species has not been previously considered, and the taxonomy of the genus has been in a state of flux for some time (Chapter 4 provides a comprehensive taxonomic review). The last review, by Sumner (1978) reduced the genus from six to one species consisting of three sub-species. Doubts have arisen about the validity of this taxonomy, due in large part to the collection of many new specimens from remote and previously inaccessible areas, as well as studies into habitat partitioning between the subspecies (Richardson and Swain 1980, Richardson and Horwitz 1988). Indeed, Dr. Alastair Richardson (School of Zoology, University of Tasmania) commissioned two allozyme electrophoretic studies into populations of the genus; the preliminary results of these studies suggested the presence of several species, however the results were never fully analysed nor published.

During 1996, I undertook a preliminary inquiry into the morphological systematics of *Parastacoides*, as an Honour's project (Hansen, 1996). The study concluded that sufficient morphological evidence existed to warrant a full review of the genus. A preliminary phylogeny of the species constructed on the basis of morphological characters suggested the presence of geographically based clades, and speciation was linked to Pleistocene glacial events. The present thesis grew from that review of

Parastacoides, and involves assessment not only of the morphology of the taxa, but also molecular studies using mitochondrial DNA (mtDNA) segments.

Specimens used in the mtDNA study were, for the most part, collected during the course of this study in order to provide fresh material for analysis. Specimens used for the rest of this project came from the School of Zoology crustacean collection. This collection contains approximately 1600 *Parastacoides* specimens. The earliest specimen in the collection was collected in December, 1939. Early additions to the collection were sporadic until the early 1970s, after which time several major collections were undertaken. These include the Australian Biological Resources Survey in the early 1970s, the Lower Gordon River Scientific Survey in the late 1970s and the Wilderness Ecosystem Baseline Studies in the late 1980s. Many of the specimens in the collection are from regions still so remote that the only practical access is by helicopter. This collection also provides an immensely useful database, never previously exploited.

Parastacoides specimens are difficult to obtain, not only because of the remote regions in which most are found, but also because each individual must be excavated from its burrow, usually a time-consuming and labour-intensive task. Richardson and Swain (1978) provide an excellent description of the burrow excavation technique, but it is worthwhile providing a brief explanation here. Once the burrow entrance has been located a clod of the substrate must be removed; a sharp spade is used to cut a square area around the entrance and this is then lifted out whole. It is important that the clod be extracted intact in order to be able to trace the burrow, both in the removed clod but also in the hole left behind. As the burrows usually ramify through the substrate there are often several shafts to trace from this stage. It is then a matter of continuing to remove clods and tracing shafts until the animal is located. This is not always possible as: a) no animal may be present in the burrow, b) tree roots or boulders may prevent further excavation or c) the shaft direction may be lost amid the mud and water filling the excavation. The time taken to remove a single specimen can range from a few minutes to an hour or more. Pitfall traps have proved to be of limited value in collecting specimens (R. Swain & A. Richardson, unpublished data), and other methods such as baited traps set in water, or opening the burrow to the water table and "roiling the water" (Hobbs 1972) have not proved to be successful.

Tasmania has several features that have the potential to make it a unique natural laboratory for the study of evolution, speciation and biogeography of its taxa. It is the most southerly, and the most mountainous part of Australia, and has remained isolated for a relatively long period of time. During this time it has been subjected to a series of major climate changes (Hill *et al.* 1999). Just as the living Tasmanian flora is a product of past events (Hill *et al.* 1999), so is the fauna. Tasmania was once part of the supercontinent Gondwana, and due to the order of rifting, was for a long time the last connection between Australia and Antarctica (Hill *et al.* 1999), facilitating the movement of plants and animals between these regions; Tasmania retains a substantial component of flora and fauna with a Gondwanan heritage.

Tasmania is one of only three southern latitude temperate land masses to have experienced regular cycles of glaciation during the Cainozoic (65 Mya to present), and it is the only one of these land masses to have remained tectonically stable during this period (Hannan *et al.* 1993). While relatively little is known concerning the Cainozoic glaciations affecting the mainland of Australia, it does appear that only minimal glaciation occurred (Kiernan 1996). Tasmania has had a complex history of multiple glacial events. Substantial glaciation has occurred in Tasmania on at least four, possibly six, occasions during the Cainozoic in Tasmania (Colhoun *et al.* 1996) (see Chapter 7.2 for details).

Tasmania provides a unique opportunity to study the response of plants and animals to changes in the Cainozoic climate without the confounding influence of geological features such as uplift. Tasmania is separated from the mainland of Australia by a substantial, albeit shallow, body of water (Bass Strait), however the regular cycle of glacial events has made Tasmania an "occasional island". Falling sea levels resulting from water locked up in ice at the height of glacial events have allowed the development of land bridges connecting Tasmania to the Australian mainland on several occasions. There is still some controversy surrounding the number of times, and the extent to which these land bridges occurred (Jackson 1999b), with different authors suggesting between five (Chappell and Shackleton 1986) and seven (Chappell 1983, Blom 1988) occasions. These land bridges potentially facilitate the dispersal of plants and animals both northward and southward (Hill *et al.* 1999).

Indeed, there is substantial similarity between the flora and fauna of northeastern and eastern Tasmania and the southeastern Australian mainland.

However the majority of Tasmania endemic flora occurs in the south and southwestern regions of the state. (Kirkpatrick and Brown 1984). Neboiss (1977) suggests that among caddis-flies, the widespread species common to the north east of Tasmania and the mainland of Australia are those which are warm-adapted Conversely, the cooler, wetter southwestern regions of Tasmania allow the persistence of cool-adapted endemic species. It appears that endemism in Tasmanian taxa is closely related to those environments which are the most different from those found in the adjacent south eastern mainland of Australia (Hill and Orchard 1999).

The southwest regions of Tasmania have remained relatively untouched by European settlers, and there is considerable debate concerning the impact of Aborigines in this area (Jones and Allen 1993). Jackson and Brown (1999) suggest that Aboriginal presence in Tasmania, during the past 40,000 years at a minimum, has increased the frequency of fire in the vegetation; the current extent of buttongrass sedgelands are the result of Aboriginal burning (Jackson 1968). Sedgelands are a major vegetation community in soutwestern Tasmania (Hill *et al.* 1993), and on the siliceous soils of southwestern Tasmania, heaths and sedgelands develop in fire-prone regions, and while Jackson and Brown (1999) suggest that it is not possible to ascribe cause and effect, they suggest that regular firing contributes to the maintenance of open heathy vegetation. However, there is evidence that while Aborigines were present in the southwestern regions of Tasmania earlier, by approximately 12 ka they had abandoned the valley habitats of the southwest. If this is the case then the vegetation has had ample time to revert to the natural communities of the region. Jarman *et al.* (1988) argue that a significant proportion of the buttongrass moorland community of southwestern Tasmania is a natural edaphic climax vegetation; sedgelands occur in waterlogged flats and depressions, and they suggest that these sites are capable of sustaining healthy sedgeland communities in the absence of fire and that it would be unlikely that these sedgeland communities would progress to forest communities. Hill *et al.* (1993) note fossil evidence to suggest that, while not the exact floristic homologues of modern buttongrass communities, a community of similar structure and ecology was present in the region tens of millions of years ago. I suggest that,

while Aboriginal firing may have at times increased the extent of sedgeland in southwestern Tasmania, the community is a natural feature of the region, and ample habitat for freshwater crayfish has existed in the region for a very long period of time. Large areas of the southwest of Tasmania are now protected by their inclusion in a World Heritage listed area, so anthropogenic impacts on invertebrate taxa in the future should be limited. This provides a unique opportunity to investigate invertebrate taxa whose distributions have remained virtually intact through a relatively long period of time, and are likely to remain so in the future.

Aims of this project

The main aim of this project was to determine the systematics and the evolutionary history of the Tasmanian endemic freshwater crayfish genus *Parastacoides*. The study had the following objectives:

1) to determine the validity of electromorphs through a complete analysis of the allozyme electrophoretic data, as well as morphological, morphometric and molecular studies,
2) to prepare a taxonomic review of the genus, and identifications keys,
3) to investigate the phylogenetic structure of the genus through allozyme, morphological and molecular studies,
4) to establish the timing of speciation events for *Parastacoides* species,
5) to develop a hypothesis for events responsible for the biogeography of the species, and
6) to examine ecological and conservation aspects arising from this review of the genus.

Thesis structure

This thesis is comprised of three major sections.

Section 1: Molecular Systematics, deals with the analysis of genetic data. This section provides data to suggest the presence of two genera, consisting of 14 species. Chapter 2 suggests evidence to support the two genera and some of the new species groups, and a proposed phylogeny, based on allozyme electrophoretic data. Analysis of mitochondrial DNA sequence data is discussed in Chapter 3.

Section 2: Morphological Systematics, is concerned with taxonomic and morphometric aspects of the new genera and species. The morphometric analysis of the new species arrangement is described in Chapter 4, and a phylogeny based on morphological characters is proposed. Chapter 5 details some taxonomic and nomenclature difficulties, but is primarily devoted to the descriptive analysis. Two new genera and several new species are described. Keys are provided.

Section 3: Biogeography, deals with issues related to the distribution of genera and species. Chapter 6.1 deals with the distribution of the genera and species, while Chapter 6.2 discusses distributional influences and habitat requirements. A general discussion of the results of the study and implications arising from these follow in Chapter 7.

Whilst I conducted the analyses of data presented in Chapter 2, electrophoretic studies were carried out by Mark Adams (Evolutionary Biology Unit, South Australian Museum) and Tom Krasnicki (School of Zoology, University of Tasmania), in two separate trials. As this work provided the foundation of much of the subsequent research, and as it had remained largely unanalysed when this project began, I felt that full analysis of the data and publication of the results was necessary for the undertaking of the remainder of the project.

Thesis presentation

With the exception of the General Introduction (Chapter 1) and the General Discussion (Chapter 7), this thesis has been written as self-contained, but inter-related, papers. Therefore, of necessity, some descriptive repetition occurs between these papers. Chapters 2 and 3.1 have been submitted to journals for review; details of the journals are presented at the start of each Chapter. The papers from these chapters are co-authored with my supervisor (Alastair Richardson) and/or my technical supervisor (Adam Smolenski). In these papers I was senior author, and responsible for data collection, data analysis and preparation of the publications. Minor formatting alterations to suit the thesis format, for example cross-referencing to other thesis Chapters, have been made, but otherwise these papers are presented as published. Chapters 3.2, 5, and 6 will be submitted to journals for publication. As a consequence, chapters follow the usual journal format in beginning with an Abstract,

followed by an Introduction, Materials and Methods section, Results section and finishing with a Discussion. Some minor formatting details, such as references to other chapters included in the thesis, will be altered to references to other papers when these chapters are submitted for publication. For the sake of consistency, and to avoid reiteration, the Reference lists have been omitted from the end of each chapter and incorporated into the general Reference list for the thesis.

Appendix A – list of molecular procedures used in the course of this thesis.

Appendix B – This paper was presented at 4th International Crustacean Congress and subsequently appeared in Crustacean Issues 12.

Appendix C – This paper was presented at 12th Symposium of the International Association of Astacology and subsequently appeared in Freshwater Crayfish 12.

Appendix D – This paper was presented at 12th Symposium of the International Association of Astacology and subsequently appeared in Freshwater Crayfish 12.

Appendix E - This paper was presented at the "The Other 99%: The Conservation and Biodiversity of Invertebrates" Conference held at the Australian Museum in Sydney, 9-12 December 1997 and subsequently appeared in The Other 99%. The Conservation and Biodiversity of Invertebrates ed by Winston Ponder and Daniel Lunney, 1999. Transactions of the Royal Society of New South Wales, Mosman. pp 210-218.

Appendix F – Taxa determination. A summary of data leading to the determination of genera and species.

Appendix G - A list of species names and codes used in this thesis

Difficulties encountered due to nomenclature problems (explained in Chapter 5) have led to the use of codes, based on location, being used to described new taxa in Chapters 2, 3, 4 and Appendices A, B, and C, of this thesis, rather than the scientific names proposed herein. These Chapters have been published, and in order to avoid

publishing *nomina nuda*, codes were substituted in place of new species names. When discussing studies from the literature I have attempted to assign the species names used there to the new species described in this thesis.

2. Substantial allozyme diversity in the freshwater crayfish *Parastacoides tasmanicus* supports extensive cryptic speciation

Hansen, B., Adams, M., Krasnicki, T. and Richardson, A.M.M.

This paper has been accepted by the journal *Invertebrate Taxonomy* for publication

Abstract

Allozyme electrophoretic studies on the freshwater crayfish genus *Parastacoides* suggests the presence of several cryptic species within this morphologically conservative taxon. Two independent allozyme studies were undertaken to assess the validity of the current taxonomy of this monotypic genus. An initial study examined 42 individuals from 10 sample sets for allozyme variation at 22 putative loci, and a subsequent study surveyed an additional 72 specimens from 20 sample sets at 16 putative loci. Both studies revealed the same general outcomes, namely (1) several instances of sympatric species diagnosable at multiple allozyme loci, (2) numerous examples of putative allopatric species with significant levels of genetic divergence (25-81%FD, 0.30-1.67 Nei D) well beyond those found between conspecific populations of any parastacid, (3) broad genetic affinities amongst putative species are inconsistent with currently-recognised morphotypes, and (4) low levels of within-population genetic variability, typical of parastacids. Although it is not possible to determine how many species are represented on the basis of these two preliminary studies, the allozyme data nevertheless indicate that an absolute minimum of 11 species and perhaps as many as 19 species are likely to be present in the genus, and indicate the need for a thorough taxonomic revision of the genus using both molecular and morphological data.

Introduction

Parastacoides is a genus of freshwater crayfish that is endemic to the island state of Tasmania. All members of this genus are strong burrowers and play a key role in the heathland ecosystems they inhabit. This is because their burrowing activity aerates the anaerobic peats (Richardson 1983, Richardson and Wong 1995), and provides habitat for a suite of other taxa

that inhabit freshwater crayfish burrows, known collectively as the pholeteros (Lake 1977, Horwitz 1989).

Despite its ecological importance, little work has been done on this genus due, in large part, to the difficulty in collecting specimens. *Parastacoides* inhabit some of the most remote and inaccessible areas in Tasmania, and each animal must be excavated from its burrow by hand, a process that may take over an hour. As a consequence, the genus is generally under-represented in museum collections given its overall distribution and abundance. This relative paucity of specimens has in turn lead to a confused taxonomic history, with between one and seven species having been recognised since the genus was erected (Clark 1936; Clark 1937; Riek 1967; Sumner 1978).

The most recent taxonomic review of *Parastacoides* (Sumner, 1978) concluded that the genus was monotypic, with three subspecies *P. tasmanicus tasmanicus*, *P. t. insignis* and *P. t. inermis*. However, the validity of this revision remains in doubt for three reasons. First, the proposed subspecies display significant overlap in their geographic distributions, a scenario inconsistent with the subspecies concept (Mayr 1969). Second, the numerical classification methodology used by Sumner (1978) and popular at that time was intended primarily as a means of assessing the relationships *between* species (Sokal and Sneath, 1963) and does not facilitate the resolution of species boundaries amongst individuals in groups where there are numerous taxa, each diagnosable by only a few characters (Hull 1984). Third, recent collections from previously unsampled regions have revealed additional morphological variability, which is suggestive of further taxonomic complexity.

Groups displaying morphological conservatism and/or subtle morphological heterogeneity are ideal candidates for molecular analysis. With this in mind, two separate allozyme studies were undertaken to investigate the broad genetic affinities of populations of *Parastacoides* in order to critically evaluate the current taxonomic treatment. The nuclear genetic markers revealed by allozyme analysis are highly appropriate for this purpose and have been successfully used to clarify taxonomic relationships in morphologically difficult groups (Richardson *et al.* 1986) including freshwater crayfish (Ziedler and Adams 1990, Horwitz *et al.* 1990, Campbell *et al.* 1994 and Austin 1996).

Materials and Methods

Specimens from populations covering all subspecies as identified by Sumner (1978) were collected by the method of excavation of burrow systems, from sites throughout Tasmania (see Figure 2.1 for detail of locations). Two separate collections were obtained and analysed independently in different laboratories and on different occasions. An initial study (referred to below as study A) was undertaken at the Evolutionary Biology Unit at the South Australian

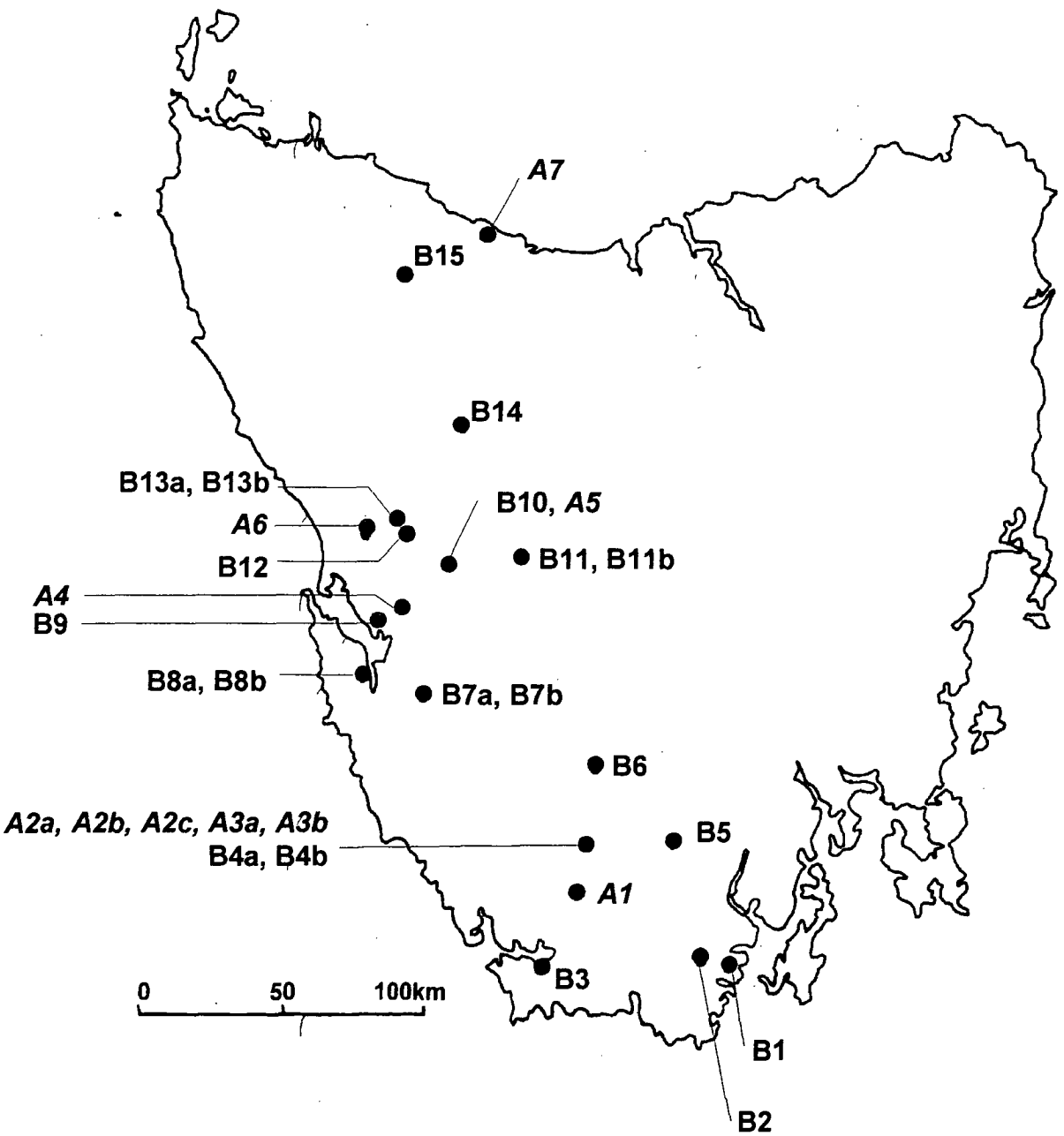


Figure 2.1. *Collection sites for both studies A (in italic text) and study B (in plain text).*

Museum, in 1983 in order to assess whether there was any genetic evidence for cryptic species amongst the six sites, and involved the collection of 10 separate freshwater crayfish samples. A follow-up study (study B) was subsequently undertaken in 1990 at the University of Tasmania with an increased sample of crayfish across a broader geographic range. Although two of the collecting localities are common to both studies, the strictly comparative nature of allozyme analysis and the unavailability of homogenates from the initial study made it impossible to integrate the two studies into a single comprehensive analysis.

The number of specimens per sample set ranged from one to six (see Table 2.1 for details). Such sample sizes are sufficient for allozyme studies of species boundaries, especially for groups that display little interpopulation variation, such as freshwater crayfish (Richardson *et al.* 1986). As the intention of these studies was taxonomic rather than phylogenetic, no outgroup was used. The study included three instances of crayfish taken from the same location (localities A2 or A3) but representing either distinct morphotypes (sample set A2a (*P.t. inermis*) versus A2b/A2c (*P.t. tasmanicus*)) or distinct habitats (A2b (slope) versus A2c (flat); A3a (slope) versus A3b (flat)). Fine-scale habitat partitioning is known to occur between *Parastacoides* subspecies (Richardson and Horwitz 1988), particularly in relation to slope and drainage. Therefore specimens from sympatric populations were collected from locations chosen to take account of local microtopographic variation. Populations termed "sympatric" were collected within 30 metres of each other. The genetic distances between sample sets are presented in Table 2.5. This study included two cases of distinct morphotypes in sympatry (sample set B4a (*P.t. inermis*) versus B4b (*P.t. tasmanicus*); B7a (*P.t. inermis*) versus B7b (*P.t. tasmanicus*)) and a further three instances of crayfish from distinctive habitats within at a locality (sample sets B8a (slope) versus B8b (flat); B11a (slope) versus B11b (flat) and B13a (slope) versus B13b (flat)).

Specimens were frozen and stored at -80°C before analysis. Electrophoresis was performed using cellulose acetate gels. Preparation and methods used for both studies are described in detail in Richardson *et al* (1986). The following enzymes or non-enzymatic proteins displayed zymograms of sufficient intensity and resolution to allow genetic interpretation in one or both studies:- fructose-bisphosphate aldolase (ALD, EC 4.1.2.13), enolase (ENOL, EC 4.2.1.11),

fructose-bisphosphatase (FDP, EC 3.1.3.11), glyceraldehyde-3-phosphate dehydrogenase (GAPD, EC 1.2.1.12), guanine deaminase (GDA, EC 3.5.4.3), aspartate aminotransferase

Table 2.1. *Details of all individuals used in the two allozyme studies. Sample sets are coded according to study (A or B) and collection locality (1-7 for study A and 1-15 for study B), with samples of morphologically distinct forms or from distinct habitats at a single locality being distinguished alphabetically (a-c in study A, a-b in study B). Collecting sites marked with an asterisk are those common to both studies.* [1] *following the subspecies recognised by Sumner (1978).+ indicates Type locality.*

Sample set	Form[1]	No	Locality	Latitude	Longitude
A1	*inermis*	6	Lake Fortuna	146°14′ E	43°08′ S
A2a*	*inermis*	3	Harlequin Hill	146°21′ E	43°58′ S
A2b*	*tasmanicus*	5	"		
A2c*	*tasmanicus*	5	"		
A3a*	*insignis*	1	"		
A3b*	*insignis*	6	"		
A4	*inermis*	5	Dacrydium Creek	145°30′ E	42°43′ S
A5*	*tasmanicus*	3	Victoria Pass	145°42′ E	42°07′ S
A6	*tasmanicus*	3	Henty River	145°31′ E	42°51′ S
A7	*tasmanicus*	5	Rubbish Tip Creek	146°04′ E	41°09′ S
B1	*tasmanicus*	4	Lune River	146°54′ E	43°29′ S
B2 +	*inermis*	2	Adamsons Peak	146°49′ E	43°19′ S
B3 +	*insignis*	4	Melaleuca	146°10′ E	43°26′ S
B4a*	*inermis*	3	Harliquin Hill	146°21′ E	43°58′ S
B4b*	*tasmanicus*	3	"		
B5	*tasmanicus*	3	Little Denison River	146°47′ E	42°58′ S
B6	*tasmanicus*	3	The Needles	146°29′ E	43°45′ S
B7a	*inermis*	4	Indiana Creek	145°41′ E	42°35′ S
B7b	*tasmanicus*	4	"		
B8a	*tasmanicus*	4	Birches Inlet	145°28′ E	42°29′ S
B8b	*tasmanicus*	4	"		
B9	*tasmanicus*	3	Macquarie Harbour	145°25′ E	42°18′ S
B10*	*tasmanicus*	4	Victoria Pass	145°42′ E	42°07′ S
B11a	*tasmanicus*	4	Mount Rufus	146°06′ E	42°08′ S
B11b	*tasmanicus*	3	"		
B12	*tasmanicus*	4	Lake Margaret	145°35′ E	42°04′ S
B13a	*tasmanicus*	4	Newton Creek	145°35′ E	42°54′ S
B13b	*tasmanicus*	4	"		
B14	*tasmanicus*	4	Lake Lilla	145°46′ E	42°39′ S
B15	*tasmanicus*	4	Takone	145°39′ E	41°11′ S

(GOT, EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), general protein (GP), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), alanine aminotransferase (GPT, EC 2.6.1.2), hexokinase (HK, EC 2.7.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), L-lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH, EC 1.1.1.37), "malic" enzyme (ME, EC 1.1.1.40), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), dipeptidase (PEP-C, EC 3.4.13.), phosphoglycerate kinase (PGK, EC 2.7.2.3), phosphoglucomutase (PGM, EC 5.4.2.2), phosphoglycerate mutase (PGAM, EC 5.4.2.1), triose-phosphate isomerase (TPI, EC 5.3.1.1), and xanthine oxidase (XO, EC 1.1.3.22). Allozymes were designated alphabetically in order of increasing electrophoretic mobility, whilst multiple loci, where present, were designated numerically, also in order of increasing electrophoretic mobility.

Framework for the taxonomic interpretation of allozyme data

Two measures of genetic divergence were employed in the analysis of the allozyme data. Percent fixed differences (%FD) were considered the most appropriate measure for assessing species boundaries (Richardson *et al.* 1986), with Nei Distance (Nei *D*, Nei 1978) being used for the construction of UPGMA dendrograms and for comparisons with other data sets (Ayala 1982; Thorpe 1982). The computer program BIOSYS-1 (Swofford & Selander, 1981) was used to calculate Nei *D* and construct dendrograms.

Several approaches have been used by those applying allozyme-based criteria to determine species boundaries amongst allopatric populations. Baverstock *et al.* (1977) showed that in rodents and *Drosophila*, populations displaying fixed differences at more than 15% of their allozyme loci inevitably represented differed biological species. This led to the formulation of the "15% rule", where allopatric populations which *differed statistically* by more than 15% (as assessed by calculating the lower 90% confidence interval for the observed data assuming a binomial distribution) revealed *prima facie* evidence of different species. Examples of the use (Baverstock *et al.* 1980) and misuse (ie. without considering confidence intervals, see Highton 1989; Frost and Hillis 1990) of this approach may be found in the literature.

Horwitz *et al.* (1990), in an allozyme study of the parastacid genus *Engaeus*, modified the "15% rule" concept to recognise three types of outcomes when comparing allopatric populations, namely (1) > 25 %FD = distinct species (without reference to confidence intervals or morphological data), (2) between 15 and 25 %FD = assess in detail the morphological distinctiveness of populations, and (3) < 15 %FD = same species. The choice of this operationally-convenient approach was considerably strengthened by the availability of a comprehensive companion study of morphology (Horwitz 1990), and was a pragmatic response to an exceptionally diverse genus comprising largely allopatric taxa.

A third approach is to use empirical criteria based on allozyme studies of a wide range of animal groups. Here the levels of genetic divergence which typically characterize populations of the same species are contrasted against those found between different species in the same genus, and between species in different genera (Ayala 1982 and Thorpe 1982). This approach results in the rough yardsticks that (1) populations belonging to the same species usually show a Nei D ≤ 0.16, (2) populations belonging to different, congeneric species generally differ in the range 0.17 < Nei D ≤ 1.00, and (3) a Nei D > 1.00 is typically associated with comparisons between separate genera (values calculated as Nei D = -ln (Nei I) using Nei I data from Ayala 1982 and Thorpe 1982).

The relationship between geographical distance and genetic distance was addressed by means of plotting one against the other in Excel and determining any correlation.

Results

Study A

A total of 22 loci were scored in this study, of which three (*Ldh*, *Gpt* and *Ald*) were monomorphic in all 42 individuals examined. Allele frequencies at the 19 polymorphic loci for the 10 sample sets are shown in Table 2.2. The number of alleles observed at each polymorphic locus varied between two and five, although most samples were mono-allelic at most loci. As a consequence, the observed mean heterozygosities were low (mean 0.036, range 0.000-0.114, Table 2.2).

Table 2.3 presents the pairwise genetic distances between all sample sets. Only one of the comparisons involving multiple samples from the same location revealed fixed differences, namely that between the "inermis" and "tasmanicus" morphotypes at locality A2 (50%FD between A2a and A2b/A2c; Table 2.3). An examination of %FDs between allopatric populations (after pooling sample sets A2b/A2c and A3a/A3b) reveals values ranging between 9% and 73%, with all but four comparisons generating a value above 31%FD, and a majority (16/27) being 50%FD or more. Thus with few exceptions, allopatric populations are readily diagnosable from one another at multiple loci. A similar pattern is evident for Nei Ds, where values range between 0.13 and 1.35, but where all but four comparisons are greater than 0.41, and a majority (16/27) above 0.75.

The broad genetic affinities of sample sets, identified according to morphotype, are displayed visually in Fig. 2.2. Two distinct groupings are evident, diverging at a large genetic distance (Nei D ~ 1.00), and revealing partial concordance with subspecific morphology. Thus all of the "tasmanicus" morphotypes cluster within one major grouping, whereas all except one of the spiny-tailed crayfish (the "inermis" and "insignis" morphotypes) fall within the other group.

Study B

Sixteen loci could be scored in study B, only one of which (*Ldh*) was monomorphic in all 72 individuals. Table 2.4 presents the allele frequencies at the 15 polymorphic loci for the 20 sample sets examined. Eleven of these loci were also used in study A. As found previously, most sample sets were fixed for a single allele, despite there being up to six alleles at a locus. This phenomenon is reflected by low levels of observed heterozygosity (mean 0.020, range 0.000 - 0.044; Table 2.4). An examination of the %FDs reveals that all five sympatric comparisons are characterized by at least one fixed difference, with values ranging between 6% (B13a and B13b) and 50% (B4a and B4b).

An examination of %FDs between allopatric sample sets (after combining sample sets B13a and B13b) reveals values ranging between 19% and 81%, with all but seven comparisons above 30%, and a majority (107/167) being 50% or more. This general pattern mirrors that found by study A, even though only half of the loci used in the earlier study were incorporated into study B. It is also supported by the Nei D values, which despite ranging from 0.21 to 1.39 amongst

Table 2.2. *Allele frequencies at 19 polymorphic loci for the 10 sample sets of study A. Frequencies are expressed as a percentage. Also shown is the observed heterozygosity (H) ± standard error (SE) for each sample set. (*** indicates no individuals in the sample set displayed activity for this enzyme)*

Locus	Allele	A1 LF	A2a HI	A2b THHH	A2c TSP	A3a INSS	A3b INSF	A4 MMC	A5 T49	A6 HR	A7 PRTC
Argk	c	100	100	-	-	100	100	-	-	-	-
	b	-	-	-	-	-	-	100	-	100	100
	a	-	-	100	100	-	-	-	100	-	-
Enol	c	92	100	100	100	100	100	100	-	-	-
	b	-	-	-	-	-	-	-	17	-	-
	a	8	-	-	-	-	-	-	83	100	100
Fdpase	c	42	17	100	100	100	100	-	-	-	-
	b	-	-	-	-	-	-	-	17	-	-
	a	58	83	-	-	-	-	100	83	100	100
Gapd	b	-	-	100	100	-	-	100	100	100	100
	a	100	100	-	-	100	100	-	-	-	-
Got-1	b	-	-	100	100	-	-	100	100	100	100
	a	100	100	-	-	100	100	-	-	-	-
Got-2	e	67	33	-	-	-	-	-	-	-	-
	d	-	-	-	-	100	100	-	-	-	-
	c	-	50	100	100	-	-	100	-	100	100
	b	33	17	-	-	-	-	-	-	-	-
	a	-	-	-	-	-	-	-	100	-	-
Gpi	c	-	-	-	-	-	-	-	17	-	-
	b	-	-	10	-	-	-	-	-	-	-
	a	100	100	90	100	100	100	100	83	100	100
Hk	b	100	83	-	-	100	100	100	17	-	-
	a	-	17	100	100	-	-	-	83	100	100
Idh	d	100	100	-	-	100	100	-	-	-	-
	c	-	-	-	-	-	-	100	100	100	-
	b	-	-	100	100	-	-	-	-	-	-
	a	-	-	-	-	-	-	-	-	-	100
Mdh1	d	-	-	-	-	-	-	100	-	100	100
	c	-	-	-	-	-	-	-	100	-	-
	b	50	-	100	100	-	-	-	-	-	-
	a	50	100	-	-	100	100	-	-	-	-
Mdh2	c	-	-	-	-	-	-	-	-	100	-
	b	100	100	-	-	100	100	100	100	-	100
	a	-	-	100	100	-	-	-	-	-	-
Mpi	c	100	100	100	40	100	100	-	100	100	100
	b	-	-	-	-	-	-	100	-	-	-
	a	-	-	-	60	-	-	-	-	-	-
PepC	d	100	100	-	-	100	100	-	-	-	-
	c	-	-	100	100	-	-	-	-	100	100
	b	-	-	-	-	-	-	100	-	-	-
	a	-	-	-	-	-	-	-	100	-	-
Pgam	d	-	-	-	-	-	-	-	-	100	100
	c	-	-	100	100	-	-	-	100	-	-
	b	100	-	-	-	100	100	100	-	-	-
	a	-	100	-	-	-	-	-	-	-	-
6Pgd	e	83	-	-	-		-	-	-	-	-
	d	17	-	100	100		10	-	-	-	-
	c	-	100	-	-	***	-	100	50	100	100
	b	-	-	-	-		90	-	-	-	-
	a	-	-	-	-		-	-	50	-	-
Pgk	e	-	-	100	80	-	-	-	-	-	-
	d	-	-	-	20	-	-	100	-	100	100

Locus	Allele	A1 LF	A2a HI	A2b THHH	A2c TSP	A3a INSS	A3b INSF	A4 MMC	A5 T49	A6 HR	A7 PRTC
	c	-	-	-	-	100	100	-	-	-	-
	b	-	-	-	-	-	-	-	100	-	-
	a	100	100	-	-	-	-	-	-	-	-
Pgm1	e	-	-	-	-	-	-	-	-	100	100
	d	30	-	-	-	-	-	-	-	-	-
	c	-	-	-	-	-	-	-	100	-	-
	b	70	100	100	100	100	92	100	-	-	-
	a	-	-	-	-	-	8	-	-	-	-
Pgm2	d	-	-	80	100	-	-	-	-	-	-
	c	-	-	20	-	-	-	100	-	100	100
	b	-	-	-	-	-	-	-	100	-	-
	a	100	100	-	-	100	100	-	-	-	-
Tpi	b	-	-	-	-	-	-	100	100	33	-
	a	100	100	100	100	100	100	-	-	67	100
Observed H		0.114	0.061	0.027	0.036	0.000	0.017	0.000	0.106	0.000	0.000
± SE		0.047	0.036	0.020	0.025	0.000	0.012	0.000	0.051	0.000	0.000

Table 2.3. *Matrix of genetic distances between sample sets for study A.*
Lower triangle = percent fixed differences; upper triangle = corrected Nei Distances.

Sample set	A1 LF	A2a HI	A2b THHH	A2c TSP	A3a INSS	A3b INSF	A4 MMC	A5 T49	A6 HR	A7 PRTC
A1	-	0.13	0.88	0.93	0.17	0.17	0.82	1.12	1.18	1.01
A2a	9	-	0.89	0.94	0.23	0.23	0.75	1.04	0.95	0.81
A2b	50	50	-	0.02	0.89	0.89	0.87	0.78	0.69	0.67
A2c	50	50	0	-	0.94	0.94	0.85	0.83	0.73	0.71
A3a	9	18	59	59	-	0.00	0.89	1.26	1.35	1.15
A3b	9	18	54	54	0	-	0.896	1.25	1.34	1.14
A4	54	50	54	54	59	59	-	0.72	0.42	0.45
A5	59	59	55	55	68	68	45	-	0.63	0.73
A6	64	55	45	45	73	73	32	41	-	0.15
A7	59	50	45	45	68	68	36	45	9	-

allopatric populations, were rarely below 0.40 (14/167) with a majority (98/167) being at least 0.70 or greater.

Figure 2.3 depicts the broad genetic affinities of all sample sets in study B. At least three highly genetically-differentiated, groupings are discernable, displaying levels of genetic divergence

(Nei Ds around 0.95 – 1.15) comparable to that distinguishing the two major groups identifiable within study A. As before, these groups do not concur exactly with the morphologically-based subspecies. The "tasmanicus" morphotype forms a cluster with two basal groups, but one population of the spiny-tailed "inermis" morphotype is found in the "tasmanicus" group.

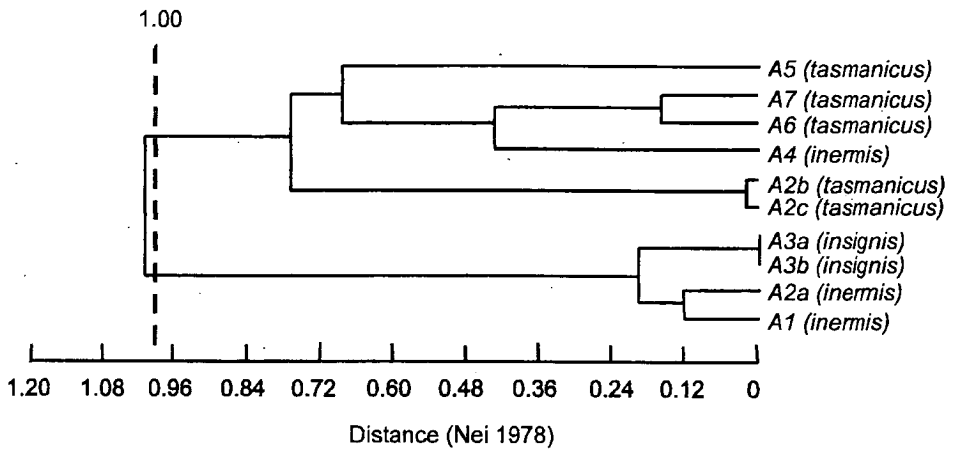


Figure 2.2. *UPGMA dendrogram based on pairwise Nei Ds between the 10 sample sets sampled in study A. Also shown is the subspecific morphotype for each sample set, sensu Sumner 1978).*

An examination of the genetic distance data for *Parastacoides* under each of the three approaches produces the same general outcome, namely that a majority of the allopatric populations sampled are likely to represent distinct species (see Table 2.4). For example, under the most conservative of approach, the "15% rule" (Baverstock *et al.* 1977), all %FDs ≥ 27% in study A (ie 6/22 loci) have a lower 90% confidence interval ≥ 16%FD, and all %FDs ≥ 31% in study B (ie 5/16 loci) have a lower 90% confidence interval ≥ 15%FD (one-tailed test of Fisher and Yates 1963, table $VIII_1$). Applying these criteria to the genetic distances data (Tables 2.3 and 2.5) results in the recognition of five species in study A (A1/A2a/A3a/A3b, A2b/A2c, A4, A6/A7, and A5) and a minimum of 11 species in study B (B1, B2/B3, B4a, B4b, B5, B7a/B7b, B8a, B8b/B9, B10, B11a, and the heterogeneous group B6/B11b/B14/B12/B13a/B13b/B15). The Horwitz *et al.* (1990) approach with its less stringent yardstick of 25%FD produces the same outcome for study A and two additional species in study B (B8b splits from B9, B6 splits from B11b/B14/B12/B13a/B13b/B15), whereas the more lenient Nei D yardsticks again recognise five species in study A but increase the number of putative species in study B to 19 (all sample sets except B13a and B13b)!

Table 2.5. *Populations representing either sympatric species, or distinct species under each of the three methods used. Sympatric species groups are indicated by brackets; commas separate the species. The symbol / indicates that the species are the same. Allopatric populations representing a species are included within brackets; species are separated by commas.*

	Study A	Study B
Sympatric species	(A2a, A2b/A2c), (A2b, A2c), (A3a, A3b)	(B4a, B4b), (B7a, B7b), (B8a, B8b), (B11a, B11b), (B13a, B13b)
Allopatric species (15% rule)	(A1/A2a/A3a/A3b), (A2b/A2c), A4, (A7/A7), A5	B1, (B2/B3), B4a, B4b, B5, (B7a/B7b), B8a, (B8b/B9), B10, B11a, (B6/B11b/B14/B12/B13a/13b/B15)
Allopatric species (Horwitz *et al.*)	(A1/A2a/A3a/A3b), (A2b/A2c), A4, (A7/A7), A5	B1, (B2/B3), B4a, B4b, B5, (B7a/B7b), B8a, B8b, B9, B10, B11a, B6, (B11b/B14/B12/B13a/13b/B15)
Allopatric species (Ayala/Thorpe)	(A1/A2a/A3a/A3b), (A2b/A2c), A4, (A7/A7), A5	B1, B2, B3, B4a, B4b, B5, B7a, B7b, B8a, B8b, B9, B10, B11a, B6, B11b, B14, B12, B13a/13b, B15

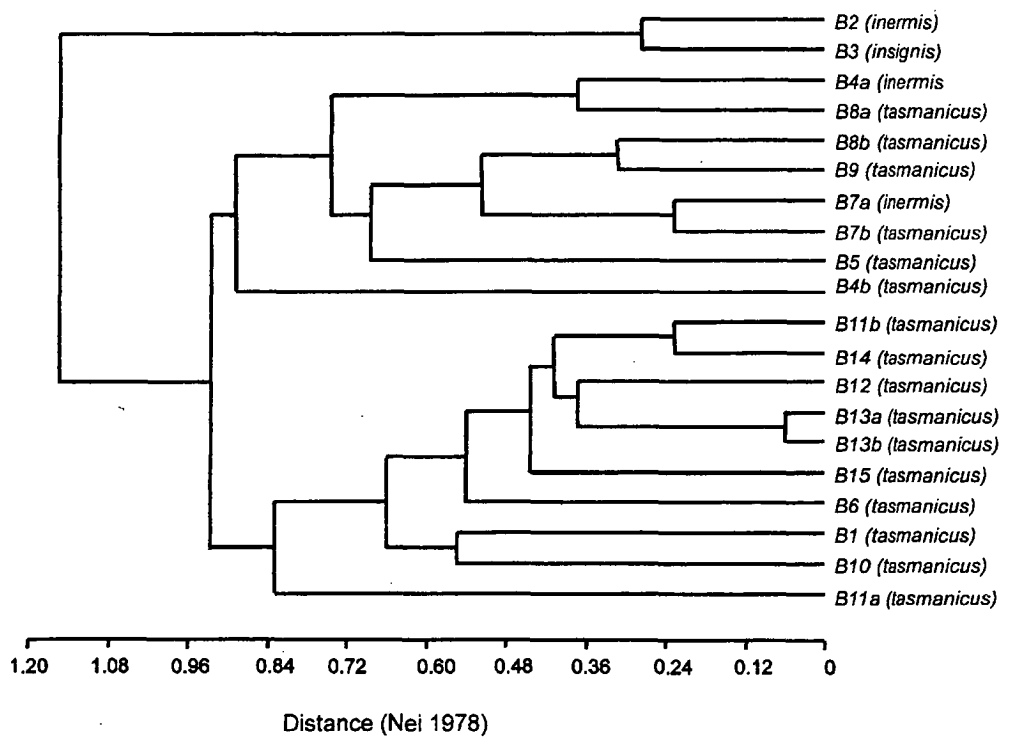


Figure 2.3. *UPGMA dendrogram based on pairwise Nei Ds between 20 sample sets sampled in study B. Also shown is the subspecific morphotype for each sample set, sensu Sumner 1978*

Table 2.4. *Allele frequencies at 15 polymorphic loci for the 20 sample sets of study B.*

Frequencies are expressed as a percentage. Also shown is the observed heterozygosity (H) ± standard error (SE) for each sample set.

Locus	Allele	B1 SET	B2 AP	B3 MEL	B4a RFI	B4b RFT	B5 LDR	B6 NEE	B7a WLI	B7b WLT	B8a BIR	B8b BIS	B9 CHT	B10 VP	B11a MR	B11b IBC	B12 LMT	B13a NCF	B13b NCS	B14 LC	B15 IRT
Enol1	c	-	100	100	100	100	100	-	-	-	100	100	100	-	-	-	-	-	-	-	-
	b	67	-	-	-	-	-	-	100	-	-	-	-	13	-	-	-	-	-	13	-
	a	33	-	-	-	-	-	100	-	100	-	-	-	87	100	100	100	100	100	87	100
Enol2	e	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	d	-	-	-	-	-	-	-	100	100	-	100	100	-	-	-	-	-	-	-	-
	c	-	100	100	100	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-
	b	-	-	-	-	-	87	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	a	100	-	-	-	-	13	100	-	-	-	-	-	100	100	100	100	100	100	100	100
Fdp	d	-	-	-	-	100	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-
	c	100	-	-	-	-	-	-	100	100	-	-	100	100	100	100	100	100	100	100	100
	b	-	-	-	100	-	100	-	-	-	100	100	-	-	-	-	-	-	-	-	-
	a	-	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gapd	d	-	100	100	-	-	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-
	c	100	-	-	-	-	100	-	100	-	50	100	100	100	-	-	100	-	-	100	100
	b	-	-	-	100	100	-	100	-	100	-	-	-	-	-	-	-	100	100	-	-
	a	-	-	-	-	-	-	-	-	-	-	-	-	-	100	100	-	-	-	-	-
Gda	e	-	-	-	-	-	-	100	100	-	-	-	-	100	-	-	-	-	100	-	-
	d	-	-	-	100	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-

Locus	Allele	B1 SET	B2 AP	B3 MEL	B4a RFI	B4b RFT	B5 LDR	B6 NEE	B7a WLI	B7b WLT	B8a BIR	B8b BIS	B9 CHT	B10 VP	B11a MR	B11b IBC	B12 LMT	B13a NCF	B13b NCS	B14 LC	B15 IRT
	c	-	-	87	-	-	-	-	-	-	-	100	100	-	100	100	100	75	-	87	-
	b	-	100	13	-	100	100	-	-	100	-	-	-	-	-	-	-	25	-	13	100
	a	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gpi	b	-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	a	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hk	f	-	-	87	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	e	-	-	13	67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	d	-	100	-	-	-	100	-	100	100	100	100	-	-	100	-	-	-	-	-	-
	c	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	13	38	-	-
	b	100	-	-	-	-	-	100	-	-	-	-	100	-	-	100	13	87	62	100	-
	a	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	87	-	-	-	100
Idh	c	-	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	-	-	-	-	-	100	67	-	-	100	100	100	-	100	100	100	-	-	-	-
	a	100	-	-	100	100	-	33	100	100	-	-	-	100	-	-	-	100	100	100	100
Mdh1	d	100	100	100	100	-	-	-	-	-	-	-	100	-	100	100	-	100	100	100	75
	c	-	-	-	-	-	-	-	100	100	100	100	-	-	-	-	-	-	-	-	25
	b	-	-	-	-	100	100	-	-	-	-	-	-	100	-	-	100	-	-	-	-
	a	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-
Mdh2	b	100	-	-	100	100	100	100	100	100	-	100	100	-	-	100	100	100	100	100	100
	a	-	100	100	-	-	-	-	-	-	100	-	-	100	100	-	-	-	-	-	-
Me	e	-	-	-	-	-	100	-	-	-	-	-	-	-	100	-	-	-	-	-	-

Locus	Allele	B1 SET	B2 AP	B3 MEL	B4a RFI	B4b RFT	B5 LDR	B6 NEE	B7a WLI	B7b WLT	B8a BIR	B8b BIS	B9 CHT	B10 VP	B11a MR	B11b IBC	B12 LMT	B13a NCF	B13b NCS	B14 LC	B15 IRT
	d	100	-	-	-	100	-	100	100	100	-	100	100	-	-	-	100	100	100	-	100
	c	-	-	-	100	-	-	-	-	-	100	-	-	100	-	-	-	-	-	-	-
	b	-	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	100	-
Mpi	b	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	87	100	100	100
	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-
Pgm1	c	-	-	100	-	-	100	-	-	-	-	-	-	-	-	-	100	100	100	100	100
	b	100	100	-	100	100	-	-	100	100	100	87	100	100	-	100	-	-	-	-	-
	a	-	-	-	-	-	-	100	-	-	-	13	-	-	100	-	-	-	-	-	-
Pgm2	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100
	c	-	-	-	100	-	-	-	-	-	100	-	100	-	-	-	100	-	-	-	-
	b	-	100	100	-	-	100	100	100	100	-	-	-	-	-	100	-	100	100	100	-
	a	100	-	-	-	100	-	-	-	-	-	100	-	100	100	-	-	-	-	-	-
Xo	d	100	100	-	100	-	100	100	100	100	100	100	100	100	-	100	-	-	-	100	100
	c	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	100	100	100	-	-
	b	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-
	a	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Observed H		0.042	0.000	0.031	0.042	0.000	0.021	0.042	0.000	0.000	0.044	0.016	0.000	0.016	0.000	0.000	0.016	0.038	0.041	0.036	0.031
± SE		0.042	0.000	0.021	0.042	0.000	0.021	0.042	0.000	0.000	0.044	0.016	0.000	0.016	0.000	0.000	0.016	0.021	0.041	0.025	0.031

Table 2.5. Matrix of genetic distances between sample sets for study B. Lower triangle = percent fixed differences; upper triangle = corrected Nei Distances.

Sample set	B1 SET	B2 AP	B3 MEL	B4a RFI	B4b RFT	B5 LDR	B6 NEE	B7a WLI	B7b WLT	B8a BIR	B8b BIS	B9 CHT	B10 VP	B11a MR-	B11b IBC	B12 LMT	B13a NCF	B13b NCS	B14 LC	B15 IRT
B1	-	1.15	1.64	0.79	0.81	1.10	0.70	0.49	0.64	1.23	0.69	0.45	0.51	0.91	0.52	0.74	0.52	0.55	0.40	0.43
B2	69	-	0.26	0.68	0.98	0.68	1.15	0.83	0.69	0.50	0.84	0.83	0.97	0.98	0.83	1.67	1.11	1.15	0.94	1.01
B3	81	19	-	0.89	1.34	0.93	1.35	1.37	1.34	0.87	1.16	0.99	1.36	0.99	0.99	0.98	0.70	0.79	0.82	1.16
B4a	56	50	56	-	0.68	0.80	0.89	0.81	0.68	0.34	0.68	0.56	0.80	1.37	0.81	1.14	0.80	0.79	0.79	0.83
B4b	56	63	69	50	-	0.82	0.76	0.83	0.58	1.14	0.70	0.83	0.82	1.39	1.16	0.97	0.76	0.76	1.12	0.81
B5	63	50	56	56	56	-	0.82	0.68	0.68	0.60	0.45	0.68	0.93	0.94	0.79	0.65	0.89	0.93	0.63	0.65
B6	44	69	75	56	50	50	-	0.64	0.52	1.20	0.83	0.72	0.77	0.86	0.49	0.70	0.42	0.34	0.52	0.62
B7a	38	56	75	56	56	50	44	-	0.21	0.74	0.38	0.47	0.55	1.16	0.69	0.82	0.68	0.56	0.54	0.53
B7b	44	50	69	50	44	50	38	19	-	0.81	0.47	0.58	0.70	0.98	0.58	0.82	0.43	0.45	0.56	0.43
B8a	69	38	56	31	69	44	69	50	56	-	0.41	0.61	0.73	0.96	0.96	1.04	1.67	1.64	1.23	1.18
B8b	50	56	69	50	50	38	50	31	38	31	-	0.29	0.83	0.80	0.70	0.68	1.01	1.14	0.82	0.77
B9	38	56	63	44	56	50	50	38	44	44	25	-	0.82	0.83	0.37	0.45	0.60	0.72	0.46	0.59
B10	38	63	75	56	56	56	50	38	50	50	56	56	-	0.70	0.70	0.59	0.83	0.68	0.58	0.46
B11a	56	63	63	75	75	56	56	69	63	63	50	56	50	-	0.47	0.69	0.71	0.81	0.71	0.85
B11b	38	56	63	56	69	50	38	50	44	63	50	31	50	38	-	0.55	0.39	0.49	0.21	0.59
B12	44	81	63	69	63	44	44	56	56	63	50	31	44	50	38	-	0.36	0.44	0.46	0.36
B13a	38	63	50	56	44	50	31	50	31	81	63	44	56	50	31	25	-	0.05	0.22	0.34
B13b	38	69	56	56	50	56	25	44	38	81	69	50	50	56	38	31	6	-	0.30	0.37
B14	31	56	56	56	63	38	38	38	38	69	56	38	44	50	19	31	19	25	-	0.28
B15	31	63	63	56	56	44	44	38	31	63	50	44	38	56	44	31	25	31	19	-

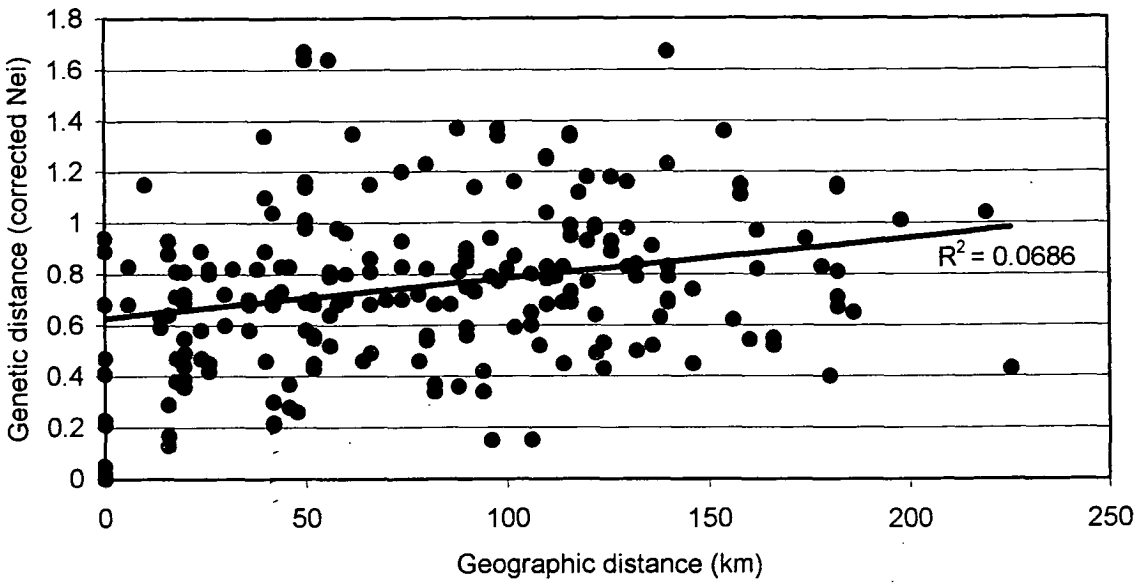


Figure 2.4. *Geographic distance plotted against genetic distance; data sets from both Study A and Study B are included. Trend line and R^2 value indicated.*

There appears to be little relationship between the geographic distance between sampled populations and genetic distance. The range of genetic distances within sympatric populations (0km geographic distance) almost covers the entire range of genetic variation at any other geographic distance (Figure 2.4).

Discussion

The two allozyme studies outlined herein both present a similar systematic picture of the genus *Parastacoides*: an unprecedented degree of genetic variation with a genus previously considered to be represented by a single species. Together they document five instances of genetically-distinct species in sympatry, numerous examples of allopatric populations which differ from all others by large genetic distances (ie > 50%FD or > 0.75 Nei D), and broad genetic groupings of samples which are at variance with the currently-recognised morphotypes (Sumner 1978). Moreover, both studies demonstrate low levels of within-population genetic variability in all taxa, an outcome that further validates the use of small sample sizes for the genetic characterization of populations of *Parastacoides*. Although neither study incorporated a large number of loci in comparison to what has been achieved on other parastacid genera (eg 36 loci in *Gramastacus*; Zeidler and Adams 1990), none of the findings listed above would be invalidated by increasing the number of loci.

Whilst the definition of species concepts is still controversial (Thorpe 1982, Allibone *et al.* 1996) the allozyme data clearly demonstrate that the genus *Parastacoides* contains multiple species. However, because of the preliminary nature of these studies it is not possible to determine the number or their boundaries. Most populations are allopatric with respect to one another, a situation which requires taxonomic decisions to be made regarding the significance of divergence (a situation which cannot be avoided for any systematic dataset, be it morphological or molecular). Where the characters under consideration are morphological, it has been common practice for systematists to make a value judgement on what represents significant taxonomic discontinuities, without explicit reference to levels of divergence or other criteria. Such an approach is certainly unacceptable when analysing molecular genetic data, just as it should also be when analysing morphological data.

We raise this issue simply to point out that the use of objective criteria when assessing allozyme data ought to be evaluated against the historical alternative, namely the subjective interpretation of morphological variation. Any such comparison should serve mainly to identify deficiencies in the latter, not problems with the former. Indeed, studies on the parastacid genus *Cherax* (Austin 1996; Austin and Knott 1996) demonstrate the inadequacies of morphological data for delineating species in this group, even where non-subjective methods of analysis are employed. Of course, both approaches are effective when dealing with sympatric forms, since all that is required in that case are multiple, genetically-determined, phenotypic differences to demonstrate that interbreeding does not occur, irrespective of the actual levels of divergence.

The inescapable conclusion from the above analyses is that, regardless of how one interprets the allozyme data, the genus *Parastacoides* comprises a large number of species (at least 11, even if all five species in study A were re-sampled in study B). It has also persisted for a considerable period of evolutionary time (as indicated by the large genetic distances). Indeed, the levels of genetic divergence found between many allopatric populations are typical of those which characterize different genera (Nei D > 1.00; Ayala 1982, Thorpe 1982). This result contrasts markedly with the morphological conservatism displayed by these crayfish and further reinforces the fact that morphological change often correlates very poorly with evolutionary time (Baverstock and Adams, 1987) and may not be a reliable indicator of

lack of it). The limitations of morphological data in the absence of a molecular genetic framework are discussed in detail elsewhere for the parastacid genus *Cherax* (Austin 1996; Austin and Knott 1996).

The levels of within-population genetic variability found in both studies were on average low (overall average H_O for 30 sample sets = 0.026), with only two sample sets displaying $H_O >$ 0.100, and only one case of a polymorphic locus displaying more than two alleles in any sample set. Indeed, $H_O = 0.000$ for over one third of all sample sets. Such an outcome is typical of freshwater crayfish both in Australia (Austin 1996; Austin and Knott 1996; Avery and Austin 1997; Campbell *et al.* 1994; Horwitz *et al.* 1990; Zeidler and Adams 1990) and in general (Nemeth and Tracey, 1979, Brown, 1980). Given the large number of species involved here for *Parastacoides* and the diversity of genera that display this phenomenon, one can only presume that it reflects some general feature(s) of the evolutionary ecology of freshwater crayfish. It may be that low levels of within-population allozyme diversity are due to various combinations of low vagility, restricted distribution, small effective population sizes and/or sporadic genetic bottlenecks for different taxa. Clearly it would be of some interest to see whether parastacids in general also show low levels of heterozygosity at microsatellite loci.

The presence of so many species within *Parastacoides,* coupled with the apparent lack of suitable morphological characters for *a priori* diagnosis, render it essential that any future systematic revision of the genus involve both molecular and morphological data. No specific conclusions can be drawn regarding the phylogenetic relationships amongst sample sets, although UPGMA dendrograms will in principle reflect the true phylogeny provided evolutionary rates are roughly the same in all lineages (Avise 1994). The predominance of short branch-lengths at higher levels of divergence suggests that the UPGMA dendrogram is unlikely to contain any specific phylogenetic information regarding individual taxa, even if rates of evolution are relatively constant throughout.

Fortunately, three findings from the allozyme studies will facilitate the use of mitochondrial DNA (mtDNA) sequence data as the molecular technique of choice for such a revision. First, the lack of evidence in either study for hybridization or introgression between sympatric species promotes confidence that the inability of mtDNA data to readily detect these

confounding phenomena is not likely to be a limitation. Second, the low levels of within-population genetic variability justify in principle the use of small sample sizes per population. Third, the ubiquity of between-population divergence reinforces the need for comprehensive geographic sampling of populations of all putative species. Together these findings, in combination with the nuclear genetic profiles themselves, will allow mtDNA sequence analysis to achieve its full potential for taxonomic, phylogenetic and phylogeographic reconstruction (Avise 1994; Hillis *et al.* 1996).

Highly genetically distinct lineages were apparent in both studies from phenetic analyses of the Nei distance data. These groupings do not appear to be geographically based (Figure 2.4), although some closely-related taxa do appear to be geographically close to one another (see Figure 2.1). For example, large genetic differences exist between the populations B1 and B2 (1.15), despite the fact that they are within 10 km of each other, whereas populations A6 and A7 show little genetic distance (0.15), despite being separated by nearly 100 km. Whilst the sampling may appear to be minimal, experience suggests that there is little chance of intermediate genetic populations between sites which may uncover a cline, thereby negating the suggestion of separate species. The lack of a strong relationship between geographic distance and genetic distance may be a reflection of the time since divergence between the species; the large genetic distances suggest ancient lineages. However, to comment on geographic distance versus genetic distance may be simplistic. Populations of *Parastacoides* are found in a wide variety of habitats in the diverse landscape found within their range; they inhabit creeks, plains (several kilometres from standing water) slopes, and ridge tops. They are to be found in elevations ranging from sea-level to high alpine conditions above 1000m; they utilise a variety of vegetation types, from buttongrass plains to rainforest. The use of mitochondrial DNA sequence data, which would allow the establishment of a timeframe for speciation events via the use of a molecular clock, may resolve the relationship more fully. Group structure appears to be at least partially related to certain morphological characteristics, in particular the presence of terminal spines on the uropod endopod. In both studies, the basal groupings identified by the UPGMA dendrograms reveal clustering of the morphotypes, in partial concordance with the subspecific morphology.

In summary, the data presented here demonstrate that *Parastacoides* is far more speciose than has been postulated in any previous systematic assessment. We stress that this is a preliminary study, however, our molecular analyses suggest the presence of a minimum of 11 and perhaps as many as 19 species; and they are consistent with the presence of more than one genus. These findings are at great variance with the current taxonomy and further highlight the advantages of molecular data for the elucidation of species boundaries in problem groups. It is clear that a systematic revision of *Parastacoides* is now urgently required, and indeed is currently underway (Hansen and Richardson in prep), involving a study of sections of the mtDNA COI and 16S genes, as well as the comparative morphology of the species groups suggested by the allozyme electrophoretic analyses, coupled with substantial geographic sampling. This review will also examine the correspondence between the findings of this study and the existing taxonomies of Clark (1939, 1941) and Riek (1951, 1967, 1969 and 1972).

Hansen, B., Smolenski, A. 2002, A preliminary examination of the molecular relationships of some crayfish species from the genus Parastacoides (Decapoda: Parastacidae), Freshwater crayfish, 13(1), 547-554

4. Morphometric variation and morphological phylogenetic relationships in the genera *Gen. 1 gen. nov.* and *Gen. 2 gen nov.*

Abstract

As part of a larger study on the systematics and evolution of the genera *Gen. 1* and *Gen. 2* a detailed morphometric study was conducted in which both linear and meristic morphological characters were assessed using multivariate statistical methods and phylogenetic techniques. The aim was to determine the usefulness of morphometric characters in determining species and generic status in a morphologically conservative taxon. Multiple discriminant analysis techniques applied to linear (shape) measurements corroborated species and generic groupings, based on earlier molecular studies, among the specimens analysed. Multidimensional scaling techniques, however, used on the meristic characters, while corroborating species groupings, indicate that these characters are less useful in discriminating between individuals belonging to some species. Phylogenetic results suggest that a phylogenetic reconstruction based on morphological characters alone in such a morphologically conservative group may prove problematic.

Introduction

Freshwater crayfish species display a degree of relative stasis in their overall morphological characteristics and behavioural patterns which suggests that their evolutionary strategy is successful, and therefore that change is not necessary (Hasiotis 1999) or desirable. Hasiotis (1993) has found freshwater crayfish body fossils, including some found within burrow systems, dating from the Upper Triassic in the United States, in which the anatomical features resemble those of modern secondary and tertiary burrowing crayfish. An Australian Paleocene fossil of a crayfish chela, with morphological features which suggest that not only is it clearly allied to, but possibly a member of the genus *Euastacus* (Sokal 1987), and a Miocene fossil of a freshwater crayfish from New Zealand (Feldmann and Pole 1994), whose overall form places it as an astacidean, and rows of spines on the chelipeds which place it within the genus *Paranephrops*, suggest that Southern Hemisphere

freshwater crayfish have also retained their basic form for millions of years, and differ little in gross morphology from extant forms. Hasiotis (1999) suggests that despite million years of evolution of the separate crayfish families, there exists an overall stasis in crayfish morphology; the genetic encoding of the crayfish *bauplan*, or generalised archetypal body plan, is both ancient and conservative.

Williamson (1987) suggests that developmental constraints may sometimes allow only a limited number of options for changes in character space. Species may find themselves in a situation where none of the limited number of choices for change may be suitable in the selective situation facing them, and stasis is therefore an effective solution. This may perhaps be an alternative explanation for the apparent morphological stasis exhibited by freshwater crayfish generally. Another explanation for this apparent morphological stasis may be the high energy costs involved in survival in often harsh or fluctuating environments. It has been suggested by Parsons (1994) that one can expect little or no evolutionary change in an organism when the energetic costs of survival are dominant; morphological stasis should occur in stressful environments, be they stable or widely fluctuating. In their study on the life history of *Parastacoides tasmanicus tasmanicus* (SPTA) Hamr and Richardson (1994) have suggested that the stress resulting from the cooler climatic conditions in Tasmania or due to the poor nutritional status of the diet (Fradd 1979), imposed constraints on the breeding capacity of females, reducing reproductive episodes to alternate years rather than annually; biennial breeding cycles are also known to occur in northern European populations of *Astacus astacus*. This suggests that, whilst the environment in which they are living has imposed constraints on them, these crayfish have adapted to, and are living in, a stable albeit stressful environment.

There can be little doubt that freshwater crayfish have also been able to survive through widely fluctuating climatic conditions. As mentioned above, the basic crayfish *bauplan* has been in existence for millions of years, indicating that both Southern Hemisphere and Northern Hemisphere freshwater crayfish have been able to survive virtually unchanged morphologically through the series of climatic changes (known as Milankovitch climate oscillations), which have occurred during that time; the eccentricity of the earth's orbit creates variation in insolation which in

turn produce large and rapid changes in temperature and precipitation every 10-100 thousand years (Dyesius and Jansson 2000).

This tendency for freshwater crayfish to be conservative in their morphology over time is reflected in the overall conservatism in the extant crayfish form. At the family level, Riek (1972) suggests that Parastacidae differ from Northern Hemisphere Astacidae mainly in secondary sexual attributes, and that some genera of Parastacidae resemble Astacidae very closely, with the exception of details of the male genitalia. Illustrations of representatives of each genus in the synopsis of the families and genera of crayfishes (Hobbs 1974) show that, aside from size and details of armature, there is great similarity in the overall body form of all crayfish.

Limited morphological variation among species may sometimes lead to difficulties in taxonomic identification; plasticity in body proportions and meristic characters paradoxically creates overlaps in these characters between and within species, and the degree of variability is such that single characters often fail to allow recognition and discrimination between species (Fitzpatrick 1963, Hamr 1992, Hobbs 1987, Hopkins 1970 and Horwitz 1990). An explanation for this plasticity may be that species distributions are inherently diverse, in that each population within the overall distribution occupies a different area within that distribution, and therefore each population experiences genetic drift and environmental pressures slightly differently (Eldredge 1995). As discussed in the chapter dealing with the taxonomy of *Gen. 1* and *Gen. 2* (Chapter 5), despite difficulty in separating some species without the use of multiple character sets, individual populations within species are often morphological distinct from other conspecific populations.

Multivariate analyses provide the opportunity to elucidate relationships between variables in large data sets, and interpretations of these data can be made which are not possible through univariate statistics alone, particularly in the exploratory stage of a study. The majority of taxonomic studies on freshwater crayfish have been morphologically based and it is perhaps surprising therefore that relatively few studies into morphometric variability, employing multivariate techniques, have been carried out. Fitzpatrick (1977) used correlation analysis to determine the most appropriate carapace measurement. Sokal (1988a, 1988b) based his studies of the

destructor group of *Cherax* on both bivariate and multivariate analyses, finding these methods useful in elucidating the species groups within the genus. In this study, the analyses of morphometric and meristic characters was undertaken in order to further clarify the taxonomic structure of the *Gen. 1* and *Gen. 2* species groups.

However, the analysis of species affinities, or phylogenetic reconstruction, based solely on morphological characters may prove to be inadequate. The phylogenetic analysis on the set of morphological characters used in this study did not clearly resolve the phylogenetic relationships between the *Gen. 1* and *Gen. 2* species groups; the three equally parsimonious trees found were not well supported when further analysed using the bootstrap technique. Other methods of phylogenetic reconstruction, such as the molecular techniques employed in Chapters 2 and 3 were more successful in resolving the phylogenetic relationships within these two genera.

Materials and Methods

Specimens for this study were those used in the taxonomic study (Chapter 5). All species were used in the phylogenetic analysis, however, LDRT was excluded from morphometric analysis part of this study as only one specimen remained for morphological examination after the four used in the allozyme study were lost. A total of 163 (including LDRT) (162 (excluding LDRT)) specimens from 28 (27) localities was included in the study (Table 4.1 lists localities, map references, allozyme populations, sympatric populations, taxon codes and number of specimens).

Selection of specimens was based on a combination of the electromorphs suggested by the allozyme electrophoretic studies mentioned above (Chapter 2), specimens suggested by the molecular studies (Chapters 2 and 3) and the taxonomic study (Chapter 5). The characters used were those used in the taxonomic review of the genera with some modification (see Chapter 5). They were chosen not only to allow variations in all dimensions of the carapace, first pereopod (chelae and carpus) and tailfan to be observed, but because they linked readily recognisable points on the anatomy and were easily repeatable. Table 4.2 provides details of these characters used for the Discriminant Function Analysis. Table 4.3 provides detail of the meristic characters.

Table 4.1. *List of localities, map references (TASMAP 1:25000 series), allozyme population (indicated by dot), sympatric species (indicated by +), and number of specimens used in this study.*

Locality	Species Code	Map Ref.	Population	Spec. Nos.
Allens Creek	ACT	8013 (855228)	•	6
Port Davey	I	8111 (323268)	(+SPTA)	6
Olga Valley	I	8012 (026545)	• (+ WCT)	6
Bramble Cove	I	8011 (184036)		6
Lake Judd	IS	8111 (495371, 490370)		6
Lake Fortuna	IS	8111 (372248)	•	6
Harlequin Hill	SPTA	8112 (475425)	• (+ SPTA)	6
Denison River	LDRT	8212 (815425)	•	1
King River	LMT	8013 (883424, 880445, 885417)	•	6
Takone	LT	8015 (858389)	•	6
Newton Creek	LT	8014 (821598)	•	6
Penguin	LT	8115 (211451)	•	6
Lake Rhona	NT	8112 (555886)		6
The Needles	NT	8112 (555692)	•	6
Vale of Rasselas	NT	8112 (452860)		6
Birches Inlet	RCT	7912 (736926)	• (+ WCT)	6
Lune River	SET	8211 (920875)	•	6
Port Davey	SPTA	8111 (324271)	(+ I)	6
Harlequin Hill	IS	8112 (475425)	• (+ IS)	6
Serpentine River	SPTB	8112 (270442)		6
Serpentine River	SPTB	8112 (374446)		6
Victoria Pass	VPT	8013 (992367)	•	6
Indiana Creek	WCT	8012 (926863)	• (+ WCT)	6
Dacrydium Creek	IS	8013 (948094)	•	6
River Derwent	LT	8113 (573067)		6
Indiana Creek	WCT	8012 (926863)	• (+ WCI)	6
Birches Inlet	WCT	7912 (736926)	• (+ RCT)	6
Olga Valley	WCT	8012 (026545)	(+ I)	6

Table 4.2. *Linear characters used in Discriminant Function analysis and Phylogenetic analysis. Means and Standard Deviations of the 163 specimens examined are indicated.*

Character (standardised to OCL)	Mean	Std. Deviation
Occipital Carapace Length (OCL)	21.4085	4.5620
Carapace Length	112.4405	3.2987
Rostrum Length	12.4405	3.2987
Rostrum Width	11.7214	1.7094
Eye Width	7.6307	2.5825
Cephalothorax Length	80.4137	5.7771
Carapace Width	51.7633	2.7854
Carapace Depth	60.0626	3.1640
Dorsolateral Boss Length	33.5047	2.7406
Chelae Length	89.7066	10.2841
Chelae Width	41.4356	5.2444
Chelae Depth	25.5170	4.2574
Dactyl Length	48.3369	6.2814
Dactyl Depth	14.4550	2.1521
Propodus Length	36.8290	5.6379
Propodus Depth	19.1009	3.3695
Carpus Length	34.6777	2.8952
Carpus Depth	28.9021	3.1416
Carpus Width	22.3285	2.5407
Pereopod2 Chelae Length	34.8022	2.4476
Pereopod2 Carpus Length	22.3977	1.4660
Pereopod2 Merus Length	40.5675	2.8782
Uropod Exopod Length	36.0765	4.2724
Uropod Endopod (2) Length	24.3681	3.3617
Uropod Endopod Length	32.5834	3.9540
Uropod Endopod Width	21.4461	3.2370

The scores for individual specimens were grouped into a species score (LDRT was included in this study): character states were established by stem-and-leaf plot analysis in SYSTAT; meristic and linear categories were imported into PATN; Gower Similarity coefficients were calculated between each pair of specimens. The meristic and linear categories were entered into a DELTA database (Dallwitz *et al.* 1993), from which a nexus file was generated for phylogenetic analysis. The Characters from Tables 4.2 and 4.3 were used together for the phylogenetic analysis.

***Table 4.3.** Characters used in Principal Co-ordinates analysis and Phylogenetic analysis*

Character	States
antennal scale lateral margin	straight, curved
antennal scale (spine strength)	strong, intermediate, weak
antennal scale (spine position)	producing or not producing from lateral margin
antennal scale (distal margin)	entire, excavate, curved
rostrum (dorsal carina)	straight, angled
rostrum (dorsal apex)	rounded, acute
rostrum (dorsal cross-section profile)	straight, concave, convex
rostrum (lateral profile)	anteriorly depressed, straight, upturned
rostrum (lateral distal apex)	margin distolaterally blunt, acute
eye orbit posterior margin	notched, entire
suborbital angle	curved, truncate
suborbital angle (curve)	shallow, deep
mandible corneous denticles	<8, 8, >8
mandible (largest corneous denticle)	3, 4, other
epistome (sagittiform anteromedian lobe)	long and narrow, short and wide
epistome (posterolateral processes)	fully divided, partially divided
epistome (posterolateral sections)	distal margin curved, straight
epistome (tubercles)	large and discrete, small and clustered
cervical groove (setae)	present, absent
great chelae (ventral margin ridge)	with, without distinct ridge extending proximal of propodus cutting surface
great chelae (lateral surface)	lateral surface tuberculate, setose-tuberculate, punctate
great chelae (adductor boss development)	strong, weak
dactyl and propodus (opposition)	meet, cross, overlap, cross and overlap
carpus (dorsal tubercles)	<4, 5, 6, >6
carpus (dorsal tubercle row)	forming, not forming distinct row
carpus (dorsomesial tubercles)	present, absent
carpus (groove impression)	present, absent
carpus (groove impression)	groove well-developed, intermediate, weak
sternal keel anterior lateral process	deep, shallow
sternal keel anterior lateral process	distally pointed, rounded
sternal keel anterior lateral process	anterior margins shorter, longer, equal to posterior margins
sternal keel anterior lateral process	meeting, not meeting centrally
sternal keel median keel	well-rounded, intermediate, narrow
sternal keel median keel	mesial ridge well-developed, not well-developed
sternal keel posterior process	deep, shallow
sternal keel posterior process	narrow, broad
sternal keel posterior process	anterior margins straight, curved
sternal keel posterior process	anterior margins shorter, longer, equal to posterior margins
sternal keel posterior process	processes meeting, not meeting centrally
uropod endopod mesial spine	multiple and terminal, single and terminal, single and not terminal, absent

Linear measurements were taken using Mitutoyo Vernier callipers to the nearest 0.02mm. Meristic characters of the specimens were scored while viewing the specimen under a Wild M5 dissecting microscope.

Morphometric analysis

Linear measurements were subjected to canonical variate analysis, also known as multiple discriminant analysis (MDA) or discriminant analysis (DISCRIM), in the SPSS package (Version 8, SPSS for Windows 1997). The main use for MDA analysis is to predict group membership from a set of variables (Tabachnick and Fidell 1989). MDA is very closely related to techniques such as MANOVA, however while techniques such as MANOVA provide an analysis of how groups differ, MDA provides an analysis of the variables which discriminate between groups (Coakes and Steed 1999). This analysis allows an assessment of whether the variability and overlap seen in the linear characters occurred primarily within or between species. Several measures of the outcome of the analysis were obtained. The first was a canonical discriminant function diagram, which illustrated the group centroids on the two functions that accounted for the majority of the variation observed. Group classifications were calculated (Table 4.4), and Wilks' Lambda was used to test the equality of the group means (Table 4.5). Eigenvalues (Table 4.6) were calculated to indicate the amount of variance explained by each function. A structure matrix was generated of the pooled within-groups correlations between the discriminating variables and the standardized canonical discriminant functions (Table 4.7). As specimens of varying size were used for this study, all length measurements were standardised by dividing by the OCL.

Morphological characters analyses

As MDA analysis is useful for continuous linear data, but not meristic data, meristic data were analysed separately using non-metric multidimensional scaling (MDS), a nonlinear technique based on a dissimilarity matrix. This analysis was carried out using the software package PATN (Belbin 1993), using Bray-Curtis similarity coefficients and the default settings suggested by Belbin (1993). MDS is designed to show graphically the relationships between objects from a table of distances between them (Manly 1994).

Linear and meristic data were also analysed together. As in Chapter 5 (Taxonomy), linear measurement character states, or categories, were established by stem-and-leaf plot analysis in SYSTAT (1989). Meristic and linear categories were imported into PATN; Gower Similarity coefficients were calculated between each pair of specimens, and cluster analysis was performed using UPGMA clustering, and a dendrogram of the results produced. Group relationships which may be relatively easy to discern in three-dimensional space may sometimes be difficult to show graphically in two-dimension space. In this instance clustering analysis was better able to show graphically the group relationships in two-dimensional space for this data set, therefore the results of the cluster analysis are shown, and not the results of the MDS.

Phylogenetic analysis

For the phylogenetic analyses, a Nexus file of the combined meristic and linear data was entered into PAUP* (version 4) (Swofford 1993). PAUP (Phylogenetic Analysis Using Parsimony) is a program designed to construct a phylogeny, or the evolutionary history of a taxon, using the principle of maximum parsimony. It achieves this by finding the tree or trees which suggest the minimum amount of evolutionary change (Swofford 1993). Maximum parsimony analysis was performed using the branch-and-bound algorithm, and the resultant trees graphed. Branches having a maximum length of zero were collapsed to yield polytomies. This option in PAUP is often used as it is preferable to have a smaller number of trees containing a number of branches with the same origin, rather than a larger number of binary trees (Swofford 1993). Of the character set used, nine characters were of the type "ordered" (Wagner), 35 characters were of the type "unordered", and all characters were given equal weighting. As all but a few of the characters were binary or had only three states, I considered it unnecessary to weight the characters, as it would be unlikely that multistate characters would influence the results unduly. There were 21 parsimony-informative characters. As mentioned, the characters were initially selected in order to aide in the identification of the established electromorphs, making the use of an outgroup invalid. Characters were chosen in order to discriminate between species within *Parastacoides*, and not in order to elicit evolutionary information in freshwater crayfish. As a result, not all character states used in this analysis are interpretable in other freshwater crayfish genera. For

example, sagittiform anteromedian lobe of the epistome in *Gen. 1* and *Gen. 2* species is always a spear shape with a single terminal point; the character states are long and narrow or short and wide, and cannot take into account a three-spined shape such as is present in *Cherax destructor*. In the absence of an outgroup therefore, all trees were rooted using the midpoint method.

Results

Morphometric analysis

Univariate analysis showed that significant differences between species existed in the linear characters (see Chapter 5). However, due to the range found within species, most of these characters were not useful diagnostically because of the degree of overlap between species (see Figure 4.1). Discriminant analysis was employed in this study to determine whether specimens could be correctly allocated to species groups, despite this overlap, when all the linear characters were analysed together. That is, was the variation between groups greater than the variation within groups. If the data were able to correctly group specimens, which of the linear characters were useful in determining species identification? Table 4.4 shows that, of the 162 specimens analysed, 95.7% of the original group cases were correctly classified using the linear data alone and excluding the meristic data. The Wilks' Lambda test for linear character significance (Table 4.5) showed a highly significant difference between the group means for all characters with the exception of the Dorsolateral Boss Length and the Pereopod 2 Carpus Length.

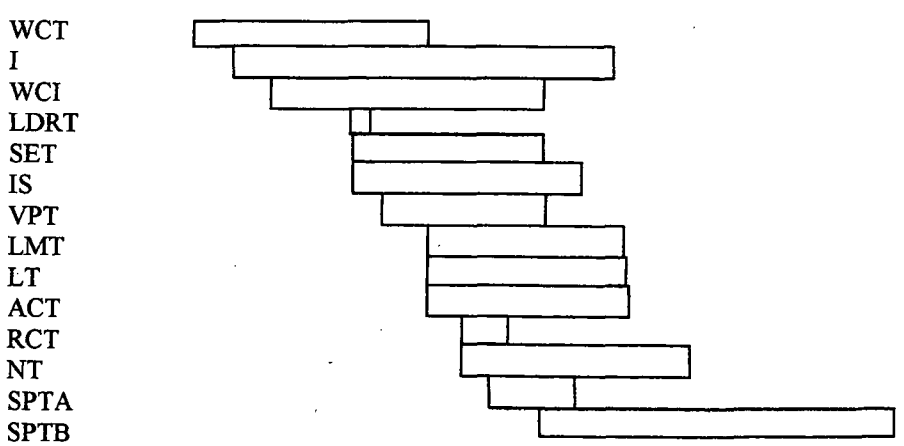


Figure 4.1. *Graphic illustrating the overlap between species in the character 'rostrum length' despite a significant difference between species possessing this character.*

The Wilks' Lambda test of equality of group means indicated that Functions 1-8 all contributed significantly to the variation (Table 4.6). Function 1 relates to rostrum length, carapace width, and a number of tailfan elements; Function 2 through 8 relate firstly to elements of the carapace and secondly to elements associated with the great chelae (see Table 4.7 for details). The eigenvalues shown in Table 4.8 indicate that 68% of the variation between the groups was explained by the first two Functions (44.3% for Function 1 and 23.7% for Function 2). The group centroids for the first two Functions (accounting for 68% of the variation) of the 13 species groups represented by 162 specimens were plotted as CDF1 in Figure 4.2. This plot suggests the presence of 13 highly-structured groups within the *Gen. 1* and *Gen. 2* species, indicating that the MDA was useful in separating the species groups based on linear measurements alone, and would suggest that despite the overlap apparent with most of the linear characters, most of the variation present is between species and not between individuals.

These species groups could further be grouped into three larger groups: (1) WCI, IS and I; (2) NT, SPTA and SPTB, species from the geographical region of northwestern Lake Pedder; and (3) the rest. This suggests that, based on the MDA, these groups are highly distinct from each other, and that the three spiny-tailed species (*Gen. 2* species) can be differentiated not only by meristic characters, but by the overall shape as well. The group centroids are clearly separated from one another along Function 2; Group 3 with scores >1, Group 2 with scores <1 > -1, and Group 1 with scores < -1. The groups are less well differentiated along Function 1, however, Groups 2 and 3 are well separated, with all Group 3 species <0, and all Group 2 species >1.

Figure 4.2 CDF2 shows the group centroid result of running DISCRIM on Group 3 species only. Figure 4.2 CDF2 shows more clearly the structure within this group, with WCT clearly distinct from the rest; WCT separates well along Function 1.

Species Group	Predicted Group Membership													Total
SPP	1	2	3	4	5	6	7	8	9	10	11	12	13	
1 ACT	6	0	0	0	0	0	0	0	0	0	0	0	0	6
2 I	0	18	0	0	0	0	0	0	0	0	0	0	0	18
3 IS	0	0	18	0	0	0	0	0	0	0	0	0	0	18
4 LMT	0	0	0	5	1	0	0	0	0	0	0	0	0	6
5 LT	0	1	0	0	17	0	0	0	0	0	0	0	0	18
6 NT	0	0	0	0	0	18	0	0	0	0	0	0	0	18
7 RCT	0	0	0	0	0	0	6	0	0	0	0	0	0	6
8 SET	0	0	0	0	0	0	0	5	0	0	1	0	0	6
9 SPTA	0	0	0	0	0	0	0	0	12	0	0	0	0	12
10 SPTB	0	0	0	0	0	0	0	0	0	12	0	0	0	12
11 VPT	0	0	0	0	0	0	0	0	0	0	5	0	1	6
12 WCI	0	0	2	0	0	0	0	0	0	0	0	16	0	18
13 WCT	0	0	0	0	0	0	0	1	0	0	0	0	17	18
1 ACT	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
2 I	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
3 IS	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
4 LMT	.0	.0	.0	83.3	16.7	.0	.0	.0	.0	.0	.0	.0	.0	100.0
5 LT	.0	5.6	.0	.0	94.4	.0	.0	.0	.0	.0	.0	.0	.0	100.0
6 NT	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	100.0
7 RCT	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	100.0
8 SET	.0	.0	.0	.0	.0	.0	.0	83.3	.0	.0	16.7	.0	.0	100.0
9 SPTA	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	100.0
10 SPTB	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	100.0
11 VPT	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	83.3	.0	16.7	100.0
12 WCI	.0	.0	11.1	.0	.0	.0	.0	.0	.0	.0	.0	88.9	.0	100.0
13 WCT	.0	.0	.0	.0	.0	.0	.0	5.6	.0	.0	.0	.0	94.4	100.0

Table 5.4. Structure matrix of group classifications indicating that 95% of the specimens were correctly identified. The top of the matrix indicates the number of specimens per group (putative species) which were identified in each group, the bottom of the matrix indcates the percent. All ACT, I, IS, NT, RCT, SPTA and SPTB specimens were assessed as belonging in fixed groups. Individual specimens from the other groups were identified as not belonging to that group.

Table 4.5. *Tests of significance of characters.*

Character	Wilks' Lambda	F	df1	df2	Sig.
Occipital Carapace Length (OCL)	0.636	7.108	12	149	0.000
Carapace Length	0.261	35.133	12	149	0.000
Rostrum Length	0.261	35.133	12	149	0.000
Rostrum Width	0.558	9.850	12	149	0.000
Eye Width	0.569	9.399	12	149	0.000
Cephalothorax Length	0.764	3.840	12	149	0.000
Carapace Width	0.418	17.256	12	149	0.000
Carapace Depth	0.564	9.604	12	149	0.000
Dorso Boss Length	0.894	1.480	12	149	0.138
Chelae Length	0.552	10.091	12	149	0.000
Chelae Width	0.647	6.778	12	149	0.000
Chelae Depth	0.789	3.317	12	149	0.000
Dactyl Length	0.448	15.292	12	149	0.000
Dactyl Depth	0.816	2.802	12	149	0.002
Propodus Length	0.516	11.656	12	149	0.000
Propodus Depth	0.440	15.797	12	149	0.000
Carpus Length	0.623	7.501	12	149	0.000
Carpus Depth	0.637	7.090	12	149	0.000
Carpus Width	0.754	4.047	12	149	0.000
Pereopod 2 Chelae Length	0.646	6.790	12	149	0.000
Pereopod 2 Carpus Length	0.905	1.303	12	149	0.222
Pereopod 2 Merus Length	0.829	2.562	12	149	0.004
Exopod Length	0.533	10.893	12	149	0.000
Endopod 2 Length	0.497	12.578	12	149	0.000
Endopod Length	0.472	13.916	12	149	0.000
Endopod Width	0.351	22.931	12	149	0.000

Table 4.6. *Tests of equality of group means.*

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 12	0.000	1126.474	168	0.000
2 through 12	0.004	803.451	143	0.000
3 through 12	0.023	559.045	120	0.000
4 through 12	0.068	396.842	99	0.000
5 through 12	0.159	270.820	80	0.000
6 through 12	0.338	160.005	63	0.000
7 through 12	0.503	101.451	48	0.000

8 through 12	0.647	64.264	35	0.002
9 through 12	0.809	31.240	24	0.147
10 through 12	0.912	13.525	15	0.562
11 through 12	0.985	2.157	8	0.976
12	0.996	0.519	3	0.915

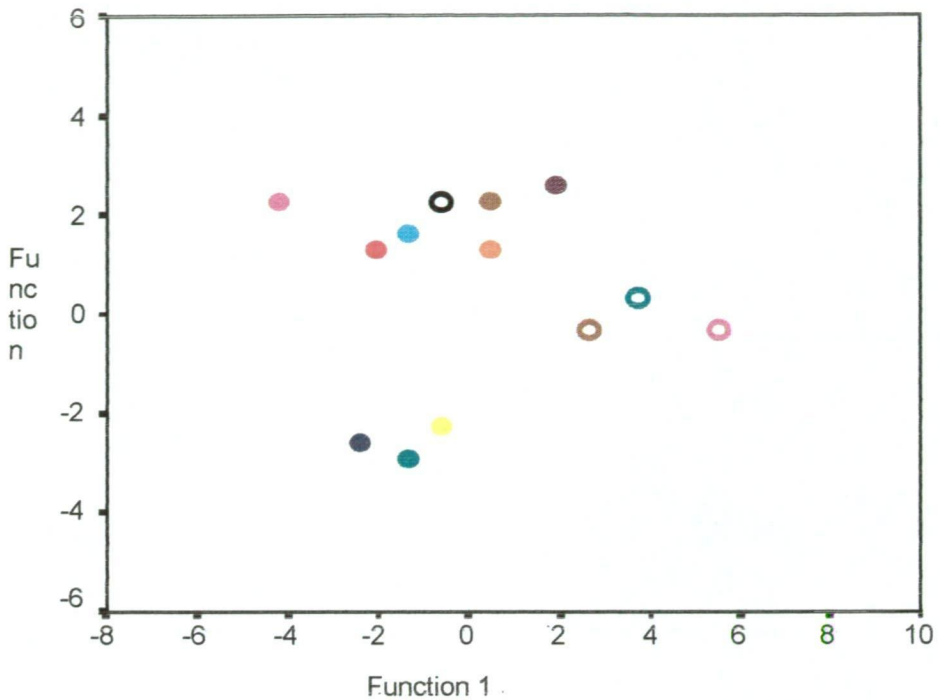
Table 4.7. *Pooled within-group correlations between discriminating variables and standardised canonical discriminant functions. The variables are ordered by absolute size of the correlation within the Function.*

Character	Function											
	1	2	3	4	5	6	7	8	9	10	11	12
Carapace L	.572	-.003	-.218	-.127	.183	.175	.269	-.377	.049	.199	.398	-.105
Rostrum L	.572	-.003	-.218	-.127	.183	.175	.269	-.377	.049	.199	.398	-.105
Exopod L	.385	.079	.037	-.007	-.045	.191	.046	-.090	-.194	-.018	-.364	.246
Endopod L	.382	-.032	-.050	-.026	.035	.148	.069	-.221	-.178	-.034	-.267	.201
Eye W	.146	-.072	-.239	-.095	.219	-.081	.202	-.110	-.061	.005	-.076	-.098
Carpus D	.141	.033	-.109	.089	-.526	.174	.220	.329	.210	-.156	-.066	-.349
Carpus L	.137	.075	-.098	-.272	-.445	.243	.291	.328	.241	.145	-.192	-.136
Rostrum W	.145	.275	.113	-.303	.350	.013	.049	-.264	-.018	.069	-.219	-.303
Carapace W	.357	.037	.240	.233	.244	.445	-.008	.255	-.221	.058	-.157	-.377
Dactyl L	.234	-.320	-.039	-.361	-.176	.068	.642	.322	.041	-.157	-.158	-.169
Propodus L	.214	-.186	-.150	-.305	-.029	.049	.525	.148	-.003	-.027	-.292	.012
Carapace D	.188	-.057	.226	.405	.211	.095	.492	-.319	.141	.172	-.354	-.291
P2 Chelae L	.299	-.126	-.163	-.171	-.001	.077	.371	.151	.014	.167	-.264	-.278
P2 Merus L	.075	-.054	-.139	.011	.023	.235	.355	.147	-.217	.172	-.131	-.215
Dactyl D	.067	-.010	-.108	.026	-.154	-.208	.329	.576	.118	-.110	.252	-.311
Chelae L	.190	-.219	-.227	-.206	-.108	.083	.434	.569	.212	.249	-.188	-.080
Propodus D	.336	-.097	.093	-.055	-.422	-.277	.226	.492	.041	.065	-.065	-.487
Chelae W	.138	-.222	-.030	-.020	-.350	-.038	.133	.371	.207	-.001	.066	-.355
P2 Carpus L	.134	.003	.047	-.097	-.116	.214	.246	.370	-.166	.045	-.195	-.105
Carpus W	.141	-.065	.052	-.034	-.290	.009	.121	.337	.172	.018	.039	-.331
Ceph L	-.059	.141	.163	.067	-.106	.131	.365	-.122	-.700	.389	.014	.092
DorsoBossL	.135	.043	-.001	.137	-.059	.037	.146	.016	-.255	-.018	-.098	-.157
Endopod2 L	.340	.104	-.064	.067	.024	-.116	-.009	-.118	-.358	-.158	-.598	.404
EndopodW	.260	-.101	-.098	-.022	.079	.182	-.004	-.242	-.198	.032	-.267	.197
OCL	-.024	.180	.325	-.095	-.325	.270	-.027	.418	.239	-.036	.101	.566
Chelae D	.082	-.139	-.056	.019	-.200	.023	.033	.255	.207	.021	.065	-.261

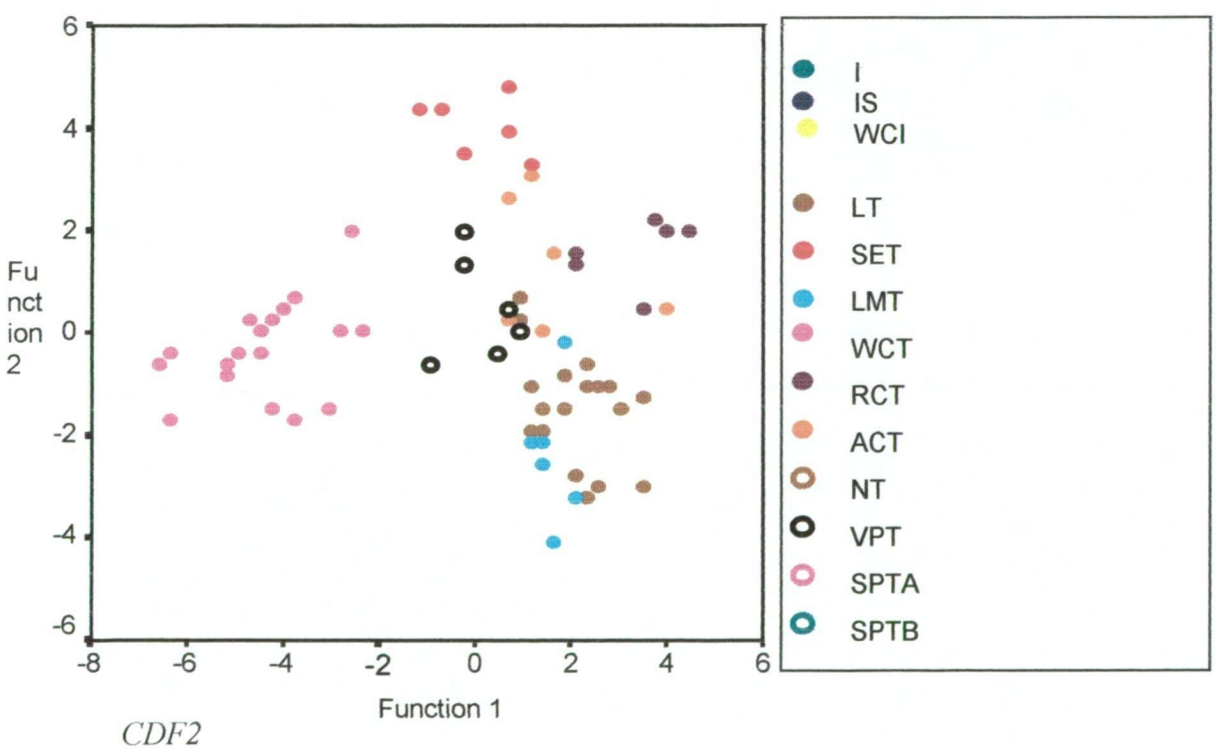
Table 4.8. *Canonical Discriminant Analysis of the linear measurement data: Function, Eigen value, percent of variance explained by the function, cumulative percent of variation explained by the functions, and the canonical correlation.*

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	7.935	44.3	44.3	0.942
2	4.244	23.7	68.0	0.900
3	2.003	11.2	79.2	0.817
4	1.350	7.5	86.8	0.758
5	1.120	6.3	93.0	0.727
6	0.487	2.7	95.8	0.572
7	0.287	1.6	97.4	0.472
8	0.251	1.4	98.8	0.448
9	0.128	0.7	99.5	0.336
10	0.080	0.4	99.9	0.272
11	0.011	0.1	100.0	0.105
12	0.004	0.0	100.0	0.059

An MDS plot of the meristic data was made using all raw meristic counts as data. PATN produced a three-dimensional solution for the data in 28 iterations, with a final stress value of 0.18. Manley (1994) suggests a stress value close to 0 is desirable, however he further suggests that the greater flexibility of non-metric scaling should enable better low-dimensional representations of the data. The result of the MDS analysis, Figure 4.3, indicates the relationship between each specimen, and not the species centroid, as in the MDA plot. In the three-dimensional space of the MDS analysis, the specimens form the species groups relatively well, though not as clearly as the linear data showed. To display the three dimensional data, three two-dimensional graphs have been plotted: Figures 4.3a-c. Allowing for apparent overlap created by the two-dimensional representation of three-dimensional space, specimens still form clear, structured groups. In Figure 4.3a, the plot produced using the first two dimensions, the groupings of specimens belonging to WCT, SET and NT are especially clear; the *Gen. 2* species form a distinct group, although the species grouping within the genus group is not as well defined. Figures 4.3b and 4.3c indicate plots produced when Dimension 1 and 3, and Dimensions 2 and 3 are plotted. Again, specimens cluster together to form relatively cohesive species groups.



CDF1



CDF2

***Figure 5.2.**. CDF1: centroid plot of the first two canonical discriminant functions using 13 species groups. CDF2: centroid plot of the first two canonical discriminant functions using seven species groups. The key applies to both Figures where appropriate*

Cluster analysis of the data using meristic characters alone does not fully resolve some of the species groups, reflecting the relatively high stress value in the MDA, and some caution should be exercised when making conclusions based solely on the meristic data. The resultant complex dendrogram extends over several pages and is not presented here. However, the cluster analysis of both meristic and linear data categories combined (see Figure 4.4) does confirm the species groupings suggested by the both MDA and MDS analyses, suggesting that despite the high stress value indicated in the MDS analysis, the results are unlikely to be highly misleading.

Phylogenetic analysis

The Branch-and-bound algorithm of PAUP produced three equally parsimonious trees with a length of 589. However, there was little support for these trees using Bootstrap and Jackknife analyses. Figure 4.5 represents the 50% Majority-rule tree resulting from the consensus of the three equally parsimonious trees. As only three trees are being compared, there would be little difference between a strict consensus tree and the majority-rule tree. However, as the majority-rule tree retains groups present in two of the three trees, the result avoids some of the uncertainties associated with polytomies that are often seen in strict consensus trees.

The separation into two clades appears in all three trees, with some variation in the minor divisions within the clades; the position of ACT and LT was the major variation between trees. The three spiny-tailed species, WCI, IS, I, do not form a monophyletic group in any tree, but are closely related sister taxa in all trees with the multiple-spined I being ancestral to the two single-spined species WCI and IS (see Chapter 5 for detailed morphological descriptions).

NT, SPTB and SPTA form a monophyletic group in all three trees, as well as in the consensus tree. LDRT, LMT, VPT. and RCT, also form a clear monophyletic group in all trees, these two groups form a monophyletic group in all trees as well.

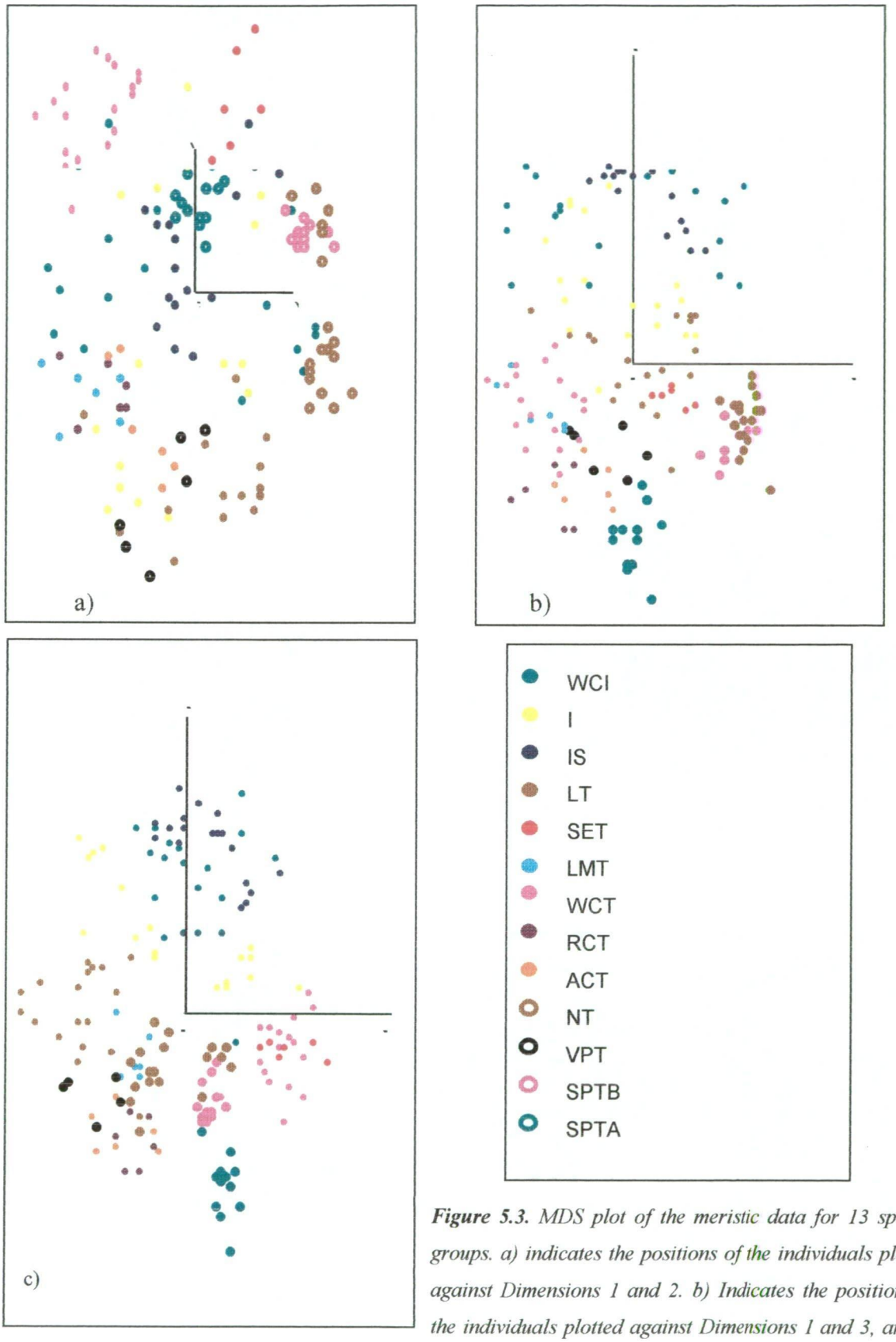


Figure 5.3. *MDS plot of the meristic data for 13 species groups. a) indicates the positions of the individuals plotted against Dimensions 1 and 2. b) Indicates the positions of the individuals plotted against Dimensions 1 and 3, and c) dimensions 2 and 3.*

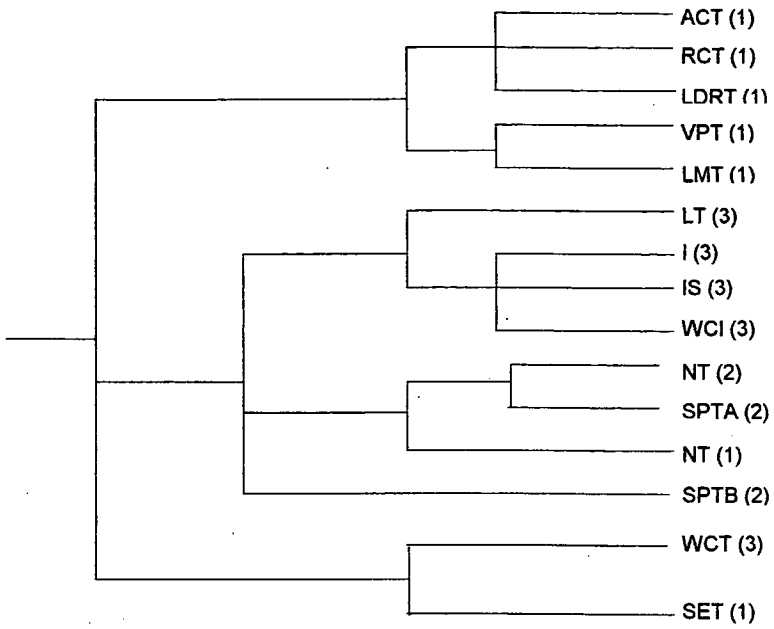


Figure 4.4. *Simplified dendrogram of the UPGMA cluster analysis of combined meristic and linear data (numbers in brackets indicate the number of populations at each terminus).*

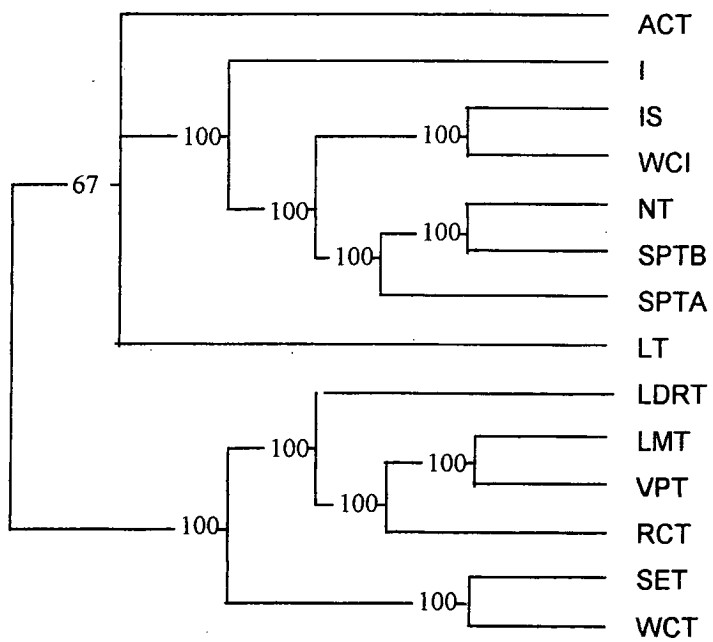


Figure 4.5. *50% majority consensus tree derived from linear and meristic data for 14 species groups.*

Discussion

Perhaps the most interesting features of the results from this study is the division of the species into three groups indicated in the MDA plot based on the linear data. In this analysis the three *Gen. 2* species were clearly grouped together (Group 1), and overall shape appears to be a reliable indicator of the generic separation of these three species. This finding corroborates the meristic data for these species groups. It appears therefore that not only are these three species distinct in their spination of the uropod endopod, but also in their overall body shape.

This grouping appears to have a geographical basis; all species are confined to an arc along the western and southern coastal regions of the state below about 42°S, from Lake Burbury in the north to the Precipitous Bluff – Mount La Perouse complex in the south (see maps in Chapter 6 - Distribution). The species belonging to the genus *Gen. 1* are divided into two distinct groups: (2) the northwestern Lake Pedder Group and (3) the rest (Figure 4.2). Interestingly, the only species possessing the distinctive 10/4 dentition morphological character, that is having the fourth of ten corneous denticles in the mandible the largest, rather than the more usual third of eight, are in group 2: NT and SPTA. The consensus phylogeny (Figure 4.5) suggests that these species are sister taxa; this dentition pattern appears to be relatively recently derived.

It does appear from these analyses that despite the overlap seen in the linear characters, and the lack of useful diagnostic characters, there are distinctive body shape differences between the species. Although the meristic data also display a degree of separation and structure between the species groups, it was not as pronounced as that seen within the linear data. It seems then that morphometric analyses, in the form of multivariate statistical analyses, can provide a useful morphological corroboration of molecular data, even in a morphologically conservative taxon where single diagnostic characters may not be available.

The phylogenetic analysis did not clearly resolve the phylogenetic relationships between the species groups; three equally parsimonious trees were found, however these were not well supported when further analysed. Despite this, some useful comments can be made on the phylogenetic analysis. From this study it appears that some monophyletic sister taxa are distributed in distinct geographically-based

regions, albeit with overlapping distributions in some areas. The two King River Valley species with restricted ranges (see Chapter 6 for map details), RCT and LMT and the other nearby species with a restricted range VPT, form a recently derived monophyletic group; speciation may be the result of peripheral isolation. Another monophyletic group of sister taxa in a small geographic area is the SPTB, NT and SPTA group, all found on the northern borders of the new Lake Pedder. As mentioned above two of these species also share a unique morphological character. However other sister taxa monophyletic groupings are harder to explain, and have no geographical basis; for example the WCT and SET. This phylogenetic reconstruction suggests that *Gen. 1* and *Gen.2* species are derived from a species without spination on the uropod endopod, and with a mandible configuration of eight corneous denticles, the third of which is the largest.

However, as has been pointed out (Marko, 1998), when sister taxa overlap in their distribution, reconstruction of the geographic background resulting in speciation will be formidable, if the modern distribution of the species is the sole basis for study. In his study of the marine snail *Nucella*, he suggests that, based only on the current distributions of some species, one would predict that speciation was triggered by vicariant events. However the molecular evidence, while not totally eliminating vicariant events, points to a combination of dispersal, peripheral isolation and climatic vicariance as determining present distribution patterns. The morphometric and phylogenetic studies presented here are a preliminary aspect of this project; clearly no real understanding of the systematics of the genera *Gen. 1* and *Gen. 2* can come from this morphological data set alone. The geographical basis for some of the monophyletic groupings suggest that peripheral isolation may have led to some speciation. However, other sister taxa groupings cannot be explained in this way, suggesting that a combination of speciation events has led to the present-day taxa. To fully understand the evolutionary history and the present biodiversity, molecular comparisons are required, as these have the potential to resolve some of the outstanding questions posed by this morphological study (see Chapters 2 and 3).

As discussed earlier, most freshwater crayfish genera display an overall conservatism of body form. In taxa which display little useful between-species morphological variation, coupled with a high degree of within-species morphological plasticity,

morphological characters alone may prove to be inadequate for systematic studies. Although some might argue from this that phylogenies based solely on morphological characters in a taxon displaying such a degree of morphological conservatism may contribute little to the understanding of *Gen. 1* and *Gen. 2* evolutionary history, I would strongly disagree. This study does, for the first time, provide a phylogenetic systematic framework within which future study and discussion can take place, and from which comparisons with molecular phylogenies can be made. Previous revisions of the genus have concentrated almost solely on the taxonomy, and little or no attempt at morphometric analysis or phylogenetic relationships between species had been attempted (Clark 1936, Clark 1939, Riek 1967, Riek 1969, Riek 1972, Sumner, 1978). This study provides an alternative hypothesis to the evolutionary history of the two genera.

5. A revision of the Tasmanian endemic freshwater crayfish genus *Parastacoides* (Crustacea: Decapoda: Parastacidae)

Abstract

The freshwater crayfish genus *Parastacoides* was last reviewed in 1978 by Sumner. In this review he synonymised several species, and reduced the genus to one species, consisting of three subspecies. However, subsequent collections of specimens from previously unsurveyed areas, as well as field studies on habitat partitioning by the subspecies, cast doubt on the taxonomic status that Sumner proposed. Allozyme electrophoretic studies of several populations were carried out (see Chapter 2); the results, suggesting the presence of several genetically distinct populations, provided the stimulus for this study. A complete revision of the genus has led to two new genera, and several new species being recognised. Keys, descriptions, diagnoses, synonymies, morphological variation and distribution maps of the genera and species are provided.

5.1Taxonomy

Introduction

All freshwater crayfish belong to the Infraorder Astacidea Latreille 1803 (Schram 1986), and representatives are found on every continent, with the exception of Antarctica, Africa and the Indian subcontinent (Hobbs 1988). Within this infraorder, freshwater crayfish are divided into two Superfamilies: the Northern Hemisphere Astacoidea and the Southern Hemisphere Parastacoidea. The main taxonomic features differentiating the two Superfamilies are that in the Astacoidea males have modified pleopods on the first abdominal segment for sperm transfer whereas the Parastacoidea do not, and the young of Astacoidea hatch and are attached to their mother by a telson thread whereas Parastacoidea young are not attached but cling by their pereopods.

The Superfamily Astacoidea is further divided into two Families: Astacidae, occurring in eastern Asia and central and eastern North America; and Cambaridae,

occurring in Europe and western North America. The greatest diversity in the Northern Hemisphere crayfish occurs in North America, with more than 300 species (Scholtz 1999). The Parastacoidea consists of one Family only, the Parastacidae. Parastacids occur in a distribution suggesting Gondwanan origins; they are found in Australia, New Guinea, New Zealand, South America and Madagascar (Hobbs 1988). The greatest diversity among the parastacids is found in southern Australia (Scholtz 1999), with over 100 species (Hobbs 1974).

Whilst there has been some debate as to the origins of freshwater crayfish, Scholtz (1993, 1998 and 1999) argues for a well supported monophyly of the Astacidea. He bases this on the presence of several apomorphic characters as well as features of the postembryonic development, when compared to other malacostracan decapods: they have an increased number of teloblasts in the ectoderm; free-living larvae are absent, and the general body shape of newly hatched young resembles that of the adults, albeit with incomplete tailfans; the first pleopods are absent; and the eyes are not yet stalked. Apomorphic features also establish the monophyly of the two Superfamilies (Scholtz, 1993, 1998 and 1999). These include differences in the hooks on chelae ends during the early stages after hatching. Astacoidea possess these on the first pereopods whereas Parastacoidea possess them on the fourth and fifth pereopods. Another apomorphic character, as mentioned above, is the sperm transfer mechanism.

Scholtz (1999) suggests that the evidence for monophyly of the Astacidea, and their world wide distribution, argues that the invasion into freshwater occurred before the breakup of the supercontinent Pangea during the Jurassic, approximately 190 to 135 millions years ago. Crayfish fossil evidence suggests that the origin of freshwater crayfish may date to the Early Carboniferous (350-320 million years ago) (Hasiotis 1999). Hasiotis (1999) and Scholtz (1999) suggest that the breakup of Pangea into Laurasia and Gondwana allowed ancestral astacids to evolve into Astacoidea and Parastacoidea.

A brief review of the taxonomic history of *Parastacoides*

This brief review of the taxonomic history is necessary in order to resolve the confused taxonomic history of the genus, and the necessity for a change in the nomenclatural status of the genera proposed in this thesis.

In 1846 Erichson reviewed the genus *Astacus*. The subgenus *Astacus (Astacus)* was raised and included one Tasmanian species, *A.(A.) tasmanicus*, based on the description of one female specimen with the type locality given as "Van Diemens Land" (later Tasmania). Erichson's brief description records this specimen as having, among other characters, "the bases of the fifth pair of legs with a gill".

Huxley (1879), in a major review of all freshwater crayfish, raised several new genera. All Australian crayfish not belonging to either *Chaeraps* (formerly *Cherax*) or *Engaeus* were placed in the new genus *Astacopsis*. Huxley mentions that Erichson had put "*Astacus madagascariensis* and some of the Australian crayfish" in the genus *Astacoides* in the 1846 monograph, however, I can only find reference to *Astacus madagascariensis* incurring this change in that paper. Therefore *Astacus (A.) tasmanicus* became *Astacopsis tasmanicus*. Von Martens (1868) and Haswell (1882) both mention *Astacoides tasmanicus* as a synonym for *Astacopsis tasmanicus* in their lists of Australian crayfish, possibly in reference to Huxley's interpretation. Ortman (1902) refers only to the genus *Astacopsis tasmanicus*. *Astacopsis tasmanicus* was mentioned by Smith (1912), as a small version of *Astacopsis franklinii*. Smith described *A. tasmanicus* species as being so similar to *A. franklinii* that that it might be considered to be only a small variety of the large form. He named localities for *A. tasmanicus* as "streams on Mount Wellington, Lake St. Clair and Zeehan". He made no mention of the type locality. In describing *A. tasmanicus* as so similar in form to *A. franklinii*, and in view of the localities he listed, Smith must have been describing either the species now placed in the genus *Astacopsis*, or a combination of *Astacopsis* and *Parastacoides* species. *Astacopsis tasmanicus* was mentioned by Faxon (1914), but again, it is uncertain to which present-day species he was referring.

In 1936, Clark undertook an extensive review of all Australian freshwater crayfish (Clark, 1936). A new genus, *Parastacoides* Clark, consisting of one species, was erected with *Astacus tasmanicus* Erichson (*nec tasmanicus* Smith) designated as the

genotype. In this review, the genus *Geocharax* was also raised, and the genus *Cherax* Erichson reviewed. Clark (1939) described two new *Parastacoides* species: *P. inermis* and *P. insignis* in a later monograph.

In the review in which the new genera were raised, Clark (1936), noted that the Type (female) of *A.(A) tasmanicus* was in the Berlin Museum, and the Type of *Cherax preissii* was missing from the Berlin Museum, and did not view the Type material. McCulloch (1917), as had von Martens (1868), noted that the type specimen of *A.(C.) preissii* was not in the Berlin Museum.

Clark's (1936) description of the genus *Parastacoides* was based on 22 specimens, 10 of which from her account, came from "near Mt. Lyell, the Type locality". Clark (1936) suggested that a character described in *A.(A.) tasmanicus* by Erichson, (the presence of gills on the 5th pair of legs), had been wrongly attributed. According to Erichson *A.(A.) tasmanicus* had gills on the 5th pair of legs, whilst *A.(Chaeraps) preissii* had no gills. Clark noted that McCulloch (1917) mentioned that all *Chaeraps* species had gills on the 5th pair of walking legs. McCulloch described *A.(C.) preissii* (from Western Australia) as synonymous with *A.(C.) intermedius* Smith (also from Western Australia); this was despite the fact that *A.(C.) intermedius* had gills on its 5th legs. Clark suggested that if one reversed the gill character, the descriptions made more sense, ie *A.(A.) tasmanicus* had no gills on the 5th pair of legs, whilst *A.(C.) preissii* had gills.

This statement appears to have caused confusion to later authors.

Riek (1951) described two further species of *Parastacoides*, *P. setosimerus* and *P. leptomerus*, but he provided no synonymies for the genus in this paper. *Parastacoides* was reviewed in full by Riek in 1967. Here he commented that the type specimen from the Berlin Museum was lost and synonymised *P. setosimerus* with *P. tasmanicus*. I suggest that Riek may have interpreted Clark's paper to read that one reversed the *type specimens* (and not only the character of gills on the 5th legs) of *A.(A.) tasmanicus* and *A.(C.) preissii*. In that case, the type specimen of *A.(A.) tasmanicus* is the one that appears to be missing from the Berlin Museum, and not that of *A.(C.) preissii*, as mentioned by von Martens and McCulloch. However, in

a later paper describing the Australian freshwater crayfish Riek (1969) wrote that the holotype of *Parastacoides tasmanicus (A. (A.) tasmanicus)* was in the Berlin Museum, but that he proposed to use the *P. setosimerus* types to describe the species, on the basis of his 1967 synonymy.

Sumner (1978) provided the most recent review of the genus. He identified three groups, *inermis* Clark *(P. inermis* Clark, *P. inermis sternalis* Riek), *insignis* Clark and *tasmanicus* Erichson (*P. tasmanicus* Erichson, *P. pulcher* Riek, *P. leptomerus* Riek), and reduced these three groups to sub-species level: *Parastacoides tasmanicus tasmanicus* Erichson, *P.t. inermis* Clark, *P.t. insignis* Clark. Sumner also suggested that the type specimen of *P. tasmanicus* (Erichson) was lost, quoting Clark's 1936 paper, and he therefore used Clark's "designated" paratype series in the Australian Museum on which to base his description of the genus. Only four specimens from this series are in now the collections of the Museum of Victoria (Ely Wallis, pers. comm.).

In 1987 Dr A.M.M. Richardson (from the School of Zoology, University of Tasmania) wrote to the Berlin Museum requesting the holotype of *A. (A.) tasmanicus* and this was provided by Dr Gruner, the then curator of Crustacea at the museum. This specimen, which had not been re-examined in any previous review of the genus, is a specimen of *Geocharax gracilis* Clark.

The distributions of *Geocharax* and *Parastacoides* do not overlap (see Figure 4.1), therefore Clark's suggestion that 10 of her specimens came from the type locality of *A. (A.) tasmanicus* near Mt Lyell cannot be correct. The specimen labelled *A. (A.) tasmanicus* in the Berlin Museum was almost certainly collected in the north-west of Tasmania, probably somewhere on the Woolnorth property of the Van Diemen's Land Company, where the collector of the specimen, Adolphus Schayer, was superintendent from 1839 to 1842 (E. Guiler, pers. comm). It remains unclear as to why Clark believed the type locality of *Parastacoides tasmanicus* (Erichson) to be near Mt. Lyell. It is almost certain that Schayer could not have collected a specimen from that region (which might have been subsequently lost or mislabelled) since at that time it was extremely inaccessible, and entirely unexplored by Europeans. The Van Diemen's Land Company also held land at Surrey Hills, south of the present

town of Burnie, which is within the present range of *Parastacoides,* but if Schayer collected material from there, it does not appear to have survived.

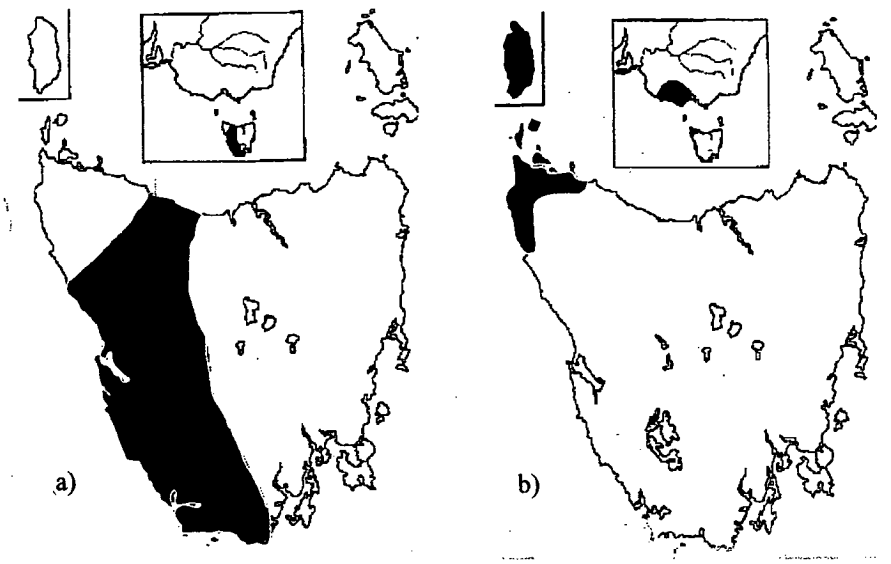


Figure 5.1. *Distributions of two Tasmania freshwater crayfish genera, a)* Parastacoides *and b)* Geocharax.

Contact with an Australian representative of the International Code of Zoological Nomenclature (ICZN) (G. Poore, Senior Curator, Crustacea, Museum Victoria) suggested that it would be illegal to continue the use of the name *Parastacoides tasmanicus* in reference to the taxa under review in this thesis. *Geocharax* has page precedence (p. 31) over *Parastacoides* (p. 48) in Clark (1936). Therefore, either both genera have the same Type species if *Geocharax gracilis* Clark, 1936 is a junior synonym of *Astacus tasmanicus* Erichson, 1846, or the Type of *G. gracilis* and *A tasmanicus* are identical, that is *G. gracilis* should now be called *G. tasmanicus* (Erichson). The older species name prevails. *Parastacoides* becomes a junior synonym of *Geocharax*. There are two resolutions possible under the ICZN rules: (1) synomymise *Parastacoides* with *Geocharax* (as they have the same Type species) or to select another species (for example *Parastacoides insignis* Clark, 1939) as Type species of a new genus which requires a new generic name; (2) select a holotype (not necessarily from Clark's material) for a species which matches Clark's concept of *P. tasmanicus* and base a new genus on this conceptual species. In both cases *P. tasmanicus* requires both a new species and a new genus name.

With material now available, (School of Zoology crustacean collection) it is possible to complete a revision of the genus; this revision has resulted in alterations to the nomenclature, the reasons for which have been explained above, and the erection of a new genus. The full synonymy of each genus, and all the species contained in the genera, is included, along with a description and distribution. A new genus, *Spinastacoides*, containing species exhibiting a terminal spine on the uropod exopod, is raised. The Type specimen proposed is one of a collection of eight syntypes of the species *Parastacoides insignis* Clark, held by the Museum of Victoria (J899) (see nomenclature point 1 above). The specimen was collected by Charles King on January 27, 1926 at New Harbour, and described by Clark (1939). In order to stabilise the status of the remaining species, I propose a new generic name; *Ombrastacoides*, and a new genotype. The new Type specimen proposed is the Allotype male of *Parastacoides leptomerus* Reik, No P.11980 (see nomenclature point 2 above) held at the Australian Museum. This specimen was collected from the Lake Lilla region in 1951, and described by Riek in 1951.

Materials and Methods

Specimens examined

Putative species suggested by a combination of the allozyme electrophoretic studies mentioned in Chapter 2, molecular studies in Chapter 3 and the morphometric study in Chapter 4, are referred to throughout by the general term "electromorphs": selection of specimens for this study was based on these electromorphs. Specimens were selected from the collection of approximately 1600 held by the School of Zoology, University of Tasmania. A range of specimens were examined to determine geographical and morphological species boundaries, and to determine if intermediates were apparent.

For the purposes of this study, three males and three females represented one population. Where possible, three populations of each electromorph were selected: 1) specimens from the population used for the allozyme electrophoresis study, as this work had established the existence of electromorphs, 2) specimens selected on the basis that the population be sympatric with at least one of the allozyme-established electromorph populations, in order to establish proof of reproductive isolation, 3) specimens selected from a population geographically isolated from the two previous

populations, preferably from a different drainage system, to establish consistency of characters across the range of the electromorph. These criteria for selection were used in order to maintain similar samples for comparison. Selection based on the above criteria was not always possible due to the lack of specimens. A total of 165 specimens from 28 localities were included in the study. Table 5.1 provides details of specimens

Table 5.1. *List of localities, map references (TASMAP 1:100 000 series), allozyme population (indicated by dot), sympatric species (indicated by +), and number of specimens used in this study.*

Locality	Map Ref.	Population	Spec. Nos.
Allens Creek	8013 (855, 228)	•	6
Port Davey	8111 (323, 268)	(+*O.huonensis*)	6
Olga Valley	8012 (26, 545)	• (+ *O. brevirostris*)	6
Bramble Cove	8011 (184, 36)		6
Lake Judd	8111 (495, 371; 490, 370)		6
Lake Fortuna	8111 (372, 248)	•	6
Harlequin Hill	8112 (475, 425)	• (+ *O. huonensis*)	6
Denison River	8212 (815,425)	•	1
King River	8013 (883, 424; 880, 445; 885, 417)	•	6
Takone	8015 (858, 389)	•	6
Newton Creek	8014 (821, 598)	•	6
Penguin	8115 (211, 451)	•	6
Lake Rhona	8112 (555, 886)		6
The Needles	8112 (555, 692)	•	6
Vale of Rasselas	8112 (452, 860)		6
Birches Inlet	7912 (736, 926)	• (+ *O. brevirostris*)	6
Lune River	8211 (920, 875)	•	6
Port Davey	8111 (324, 271)	(+ *S. insignis*)	6
Harlequin Hill	8112 (475, 425)	• (+ *S. inermis*)	6
Serpentine River	8112 (270, 442)		6
Serpentine River	8112 (374, 446)		6
Victoria Pass	8013 (992,367)	•	6
Indiana Creek	8012 (926, 863)	• (+ *O. brevirostris*)	6
Dacrydium Creek	8013 (948, 94)	•	6
River Derwent	8113 (573, 67)		6
Indiana Creek	8012 (926, 863)	• (+ *S. catinipalma*)	6
Birches Inlet	7912 (736, 926)	• (+ *O. asperrimanus*)	6
Olga Valley	8012 (26, 545)	(+ *S. insignis*)	6

used. Only adults were used in the course of the study; following Sumner (1978), specimens with a total length of less than 20mm were considered to be juvenile, and therefore excluded from the study.

All existing type specimens were examined.

Character selection

Many characters were adapted from previously published descriptions of the genus, (Clark, 1936, 1939, Riek, 1951, 1967 and Sumner, 1978). Reviews of other freshwater crayfish genera were also examined for taxonomically useful characters not previously used for *Parastacoides*, for example the use of mandible dentition in the Madagascan freshwater crayfish genus *Astacoides* by Hobbs (1987). Specimens were also examined for new characters. Terminology used in the descriptions of the morphology generally follow recent revisions of freshwater crayfish (for example Hobbs, 1987 and Horwitz, 1990). However, some features of the morphology required further detailed explanation, and these features have therefore been divided into several components or characters, even though they are single unified structures (Figures 5.2 and 5.3).

A full list of characters used appears as Table 5.2.

Table 5.2. *List of characters, their states and origins used in this study.*

Char. No.	Character (+ states)	Character derived from:	Character used in key with Figure references
1	Antennal Scale (lateral margin) 1.straight, 2.curved	new character	1) 5.13G, 5.18G 2) 5.7G, 5.10G, 5.14G
2	Antennal Scale (spine strength) 1.strong, 2.weak	new character	1) 5.7G, 5.8G, 5.14G 2) 5.9G, 5.12G
3	Antennal Scale (spine location) 1.forming lateral margin, 2.not forming lateral margin	new character	1) 5.7G, 5.8G, 5.9G 2) 5.18G, 5.20G
4	Antennal Scale (distal margin) 1.entire, 2.emarginate, 3.curved	new character	
5	Rostrum Length ((Carapace length – OCL)/OCL) 1.short (<10% OCL), 2.long (>10% OCL)	adapted from Clark 1939	
6	Rostrum width (Rostrum width/Rostrum length) 1.broad (>100% Rostrum length),	adapted from Riek 1951, 1967	1) 5.10B, 5.12B 2) 5.14B

	2.narrow (<100% Rostrum length)		
7	Rostrum (dorsal carina) 1.straight, 2.angled	new character	1) 5.7B, 5.9B, 5.13B 2) 5.8B, 5.11B, 5.16B
8	Rostrum (dorsal apex) 1.rounded, 2.acute	adapted from Clark 1936, 1939; Riek 1951, 1967	1) 5.9B, 5.10B, 5.12B 2) 5.7B, 5.8B, 5.11B
9	Rostrum (cross-section profile) 1.flat, 2.concave, 3.convex	new character	1) 5.10B 2) 5.11B 3) 5.13B
10	Rostrum (lateral profile) 1.anteriorly depressed, 2.straight, 3.upturned	new character	
11	Rostrum (distolateral margin) 1.blunt, 2.acute	new character	
12	Eye (Eye width/OCL) 1.small (<5% OCL), 2.large (>5% OCL)	adapted from Riek 1967	
13	Eye (orbit posterior margin) 1.notched, 2.entire	new character	
14	Eye (suborbital angle) 1.curved, 2.truncate	new character	
15	15. Eye (curved suborbital angle) 1.curve shallow, 2.deep	as 14	
16	Mandible (number of corneous denticles) 1.fewer than eight, 2.eight, 3.more than eight	adapted from Hobbs 1987	1) 5.9H 2) 5.7H, 5.9H, 5.10H, 5.12H, 5.13H, 5.14H, 5.15H, 5.16H, 5.17H, 5.18H, 5.19H, 5.20H 3) 5.8H, 5.11H
17	Mandible (largest corneous denticle) 1.number three, 2.number four, 3.other	as 16	1) 5.7H, 5.9H, 5.10H, 5.12H, 5.13H, 5.14H, 5.15H, 5.16H, 5.17H, 5.18H, 5.19H, 5.20H 2) 5.8H, 5.11H
18	Epistome (sagittiform anteromedian lobe) 1.long, narrow, 2.short, wide	adapted from Clark 1936	
19	Epistome (posterolateral processes) 1.fully divided, 2.partially divided	new character	1) 5.7I, 5.9I, 5.11I, 5.13I 2) 5.8I, 5.10I, 5.16I
20	Epistome (distal margin) 1.curved, 2.straight	new character	1) 5.8I, 5.9I, 5.12I, 5.14I 2) 5.7I, 5.11I, 5.16I
21	Epistome (tubercles) 1.large, discrete, 2.small, clustered	new character	
22	Cephalothorax length (Cephalothorax length/OCL) 1.short (<80% OCL), 2..long (>80% OCL)	adapted from Riek 1967	
23	Carapace width (carapace width/OCL) 1.narrow (<50% OCL), 2.wide (>50% OCL)	as 22	1) 5.16B 2) all others
24	Carapace depth (carapace depth/OCL) 1.shallow (<60% OCL), 2.deep (>60% OCL)	as 22	
25	Dorsolateral boss distance from eye orbit (distance of dorsolateral boss from posterior carina of eye orbit/OCL)	new character	

	1.close to eye orbit (<32% OCL), 2. distant from eye orbit (>32% OCL)		
26	Dorsolateral boss placement on carapace (distance between dorsolateral bosses/carapace width) 1.high (<60% OCL), 2.low (>70% OCL)	new character	1) 5.15B 2) 5.17B
27	Cervical groove (impression) 1.very deep, 2.impression deep, 3.impression shallow	new character	
28	Cervical groove (dorsal view) 1.rounded U, 2.rounded notched U	new character	
29	Cervical groove (lateral setae) 1.present, 2.absent	new character	1) 5.10A, 5.13A, 5.18A 2) 5.7A, 5.8A, 5.9A
30	Great chelae length (chelae length/OCL) 1.short (<100% OCL), 2.long (>100% OCL	adapted from Newcombe 1970	
31	Great chelae width (chelae width/chelae length) 1.narrow (<50% chelae length), 2.wide (>50% chelae length)	new character	
32	Great chelae depth (chelae depth/chelae width) 1.palm shallow (<60% chelae width), 2.palm deep (>60% chelae width)	new character	
33	Great chelae ventral margin ridge 1.extending proximal of propodus cutting surface, 2.not extending proximal of propodus cutting surface	new character	
34	Great chelae (lateral surface) 1.tuberculate, 2.setose-tuberculate, 3.punctate	new character	
35	Great chelae (adductor boss development) 1.strong, 2.weak	new character	1) 5.18D 2) 5.20D
36	Great chelae dactyl length (dactyl length/chelae length) 1.short (<50% chelae length), 2.long (>50% chelae length)	adapted from Newcombe 1970	
37	Great chelae dactyl depth (dactyl depth/dactyl length) 1.thin (<30% dactyl length), 2.thick (>30% dactyl length)	new character	
38	Great chelae propodus length (propodus length/chelae length) 1.short (<40% chelae length), 2.long (>40% chelae length)	new character	
39	Great chelae propodus depth (propodus depth/propodus length) 1.shallow (<50% propodus length), 2.deep (>50% propodus length)	new character	
40	Great chelae (dactyl and propodus opposition) 1.meeting, 2.crossing, 3.overlapping, 4.crossing and overlapping	new character	1) 5.7C, 5.8C, 5.11C 2) 5.9C 3) 5.10C 4) 5.12C, 5. 14C
41	Great chelae carpus length (carpus length/carpus width) 1.short (<110% CW), 2.long (>110% CW)	new character	

42	Great chelae carpus depth (carpus depth/carpus length) 1.narrow (<85% CL), 2. wide (>85% CL)	new character	
43	Great chelae carpus width (carpus depth/carpus width) 1.shallow (<130% CD), 2.wide (>130% CD)	new character	
44	Great chelae carpus (number of dorsal tubercles) 1.fewer than four, 2.four, 3.five, 4.six, 5.more than six	new character	
45	Great chelae carpus (dorsal tubercles) 1.forming row, 2.not forming row	adapted from Riek 1967	
46	Great chelae carpus (dorsomesial tubercle row) 1.present, 2.absent	new character	
47	Great chelae carpus (groove impression) 1.present, 2.absent	new character	1) all others 2) 5.10C
48	Great chelae carpus (groove impression if present) 1.well-developed, 2. weak	new character	
49	Pereopod 2 chelae length (P2chelae/(P2chelae+P2carpus+P2merus)) 1.short (<35% of pereopod), 2. long (>35% of pereopod)	new character	
50	Pereopod 2 (P2chelae+P2carpus+P2merus) 1.short (<95%), 2.long (>95%)	new character	
51	Sternal keel (anterior lateral process) 1.broad, 2.narrow	new character	1) 5.13J, 5.15J 2) 5.7J, 5.8J, 5.10J
52	Sternal keel (anterior lateral process) 1.distally pointed, 2.distally rounded	new character	
53	Sternal keel (anterior lateral process anterior margins) 1.shorter than posterior margins, 2.longer than posterior margins, 3.anterior and posterior margins equal	new character	
54	Sternal keel (anterior lateral process) 1.meeting centrally, 2.not meeting centrally	new character	
55	Sternal keel median keel 1.well-rounded, 2.intermediate, 3.narrow	adapted from Riek 1967	1) 5.7J, 5.12J, 5.13J 2) 5.8J, 5.9J, 5.10J
56	Sternal keel median keel (mesial ridge) 1.well-developed, 2.not well-developed	new character	1) 5.9J, 5.10J, 5.11J 2) 5.7J, 5.8J, 5.12J
57	Sternal keel (posterior process) 1.deep, 2.shallow	new character	
58	Sternal keel (posterior process) 1.narrow, 2.broad	new character	
59	Sternal keel (posterior process anterior margins) 1.straight, 2.curved	new character	
60	Sternal keel (posterior process anterior margins) 1.shorter than posterior margins, 2.longer than posterior margins, 3.anterior and posterior margins equal	new character	

61	Sternal keel (posterior process) 1.meeting centrally, 2.not meeting centrally	new character	
62	Uropod (endopod mesial spine) 1.present, 2.absent	adapted from Clark 1939; Riek 1967	1) 5.7E, 5.8E, 5.9E, 5.14E, 5.15E, 5.16E, 5.17E, 5.18E, 5.19E, 5.20E 2) 5.10E, 5.11E, 5.12E, 5.13E,
63	Uropod (endopod mesial spine) 1.terminal, 2.non-terminal	adapted from Clark 1939; Riek 1967	1) 5.18E, 5.19E, 5.20E 2) all others
64	Uropod (endopod mesial spine) 1.single, 2.multiple	adapted from Clark 1939; Riek 1967	1) 5.19E 2) 5.18E, 5.20E
65	Uropod Endopod 1. narrow (<65%endopod length), 2. wide (> 65% endopod length)	new character	
66	Telson length 1.short (<40%OCL), 2. long (>40% OCL)	new character	

Abbreviations in Descriptions

The following abbreviations have been used in the species descriptions. The standard dimension used to characterise each specimen was the occipital carapace length (OCL). The mandible dentition formula consists of the number of corneous denticles followed by the number of the largest denticle, for example 10-4 (See Figure 5.5).

The names of the collectors have been abbreviated as follows: M. Boyle (MB), A. Brettingham-Moore (AB-M), D. Coleman (DC), A. Fleming (AF), T. Fletcher (TF), I. Growns (IG), J. Hickman (JLH), V. Hickman (VVH), P. Hamr (PH), R. Holmes (RH), P. Horwitz (PHJH), P. Humphries (PH), B. Knott (BK), R. Mawbey (RBM), J. Ong (JO), C. Reid (CR), A.M.M. Richardson (AMMR), D. Ritz (DAR), D. Sander (DS), P. Suter (PS), R. Swain (RS), W. Walker (WW), I. Wilson (ISW).

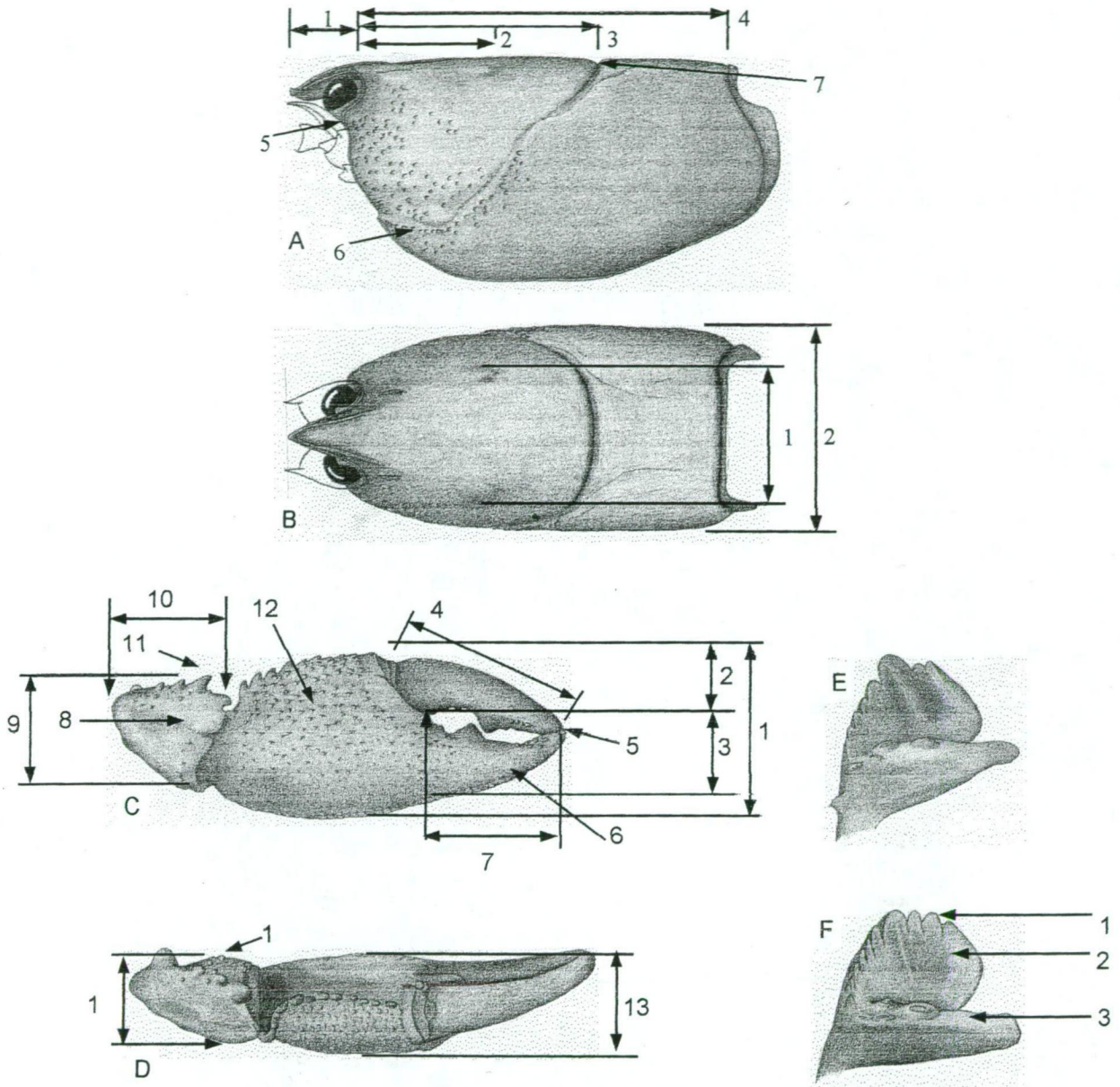


Figure 52. *Some characteristics of the carapace showing lateral view (A) and dorsal view (B) : 1, rostrum length; 2, dorsolateral boss distance from eye; 3, cephalothorax length; 4, OCL; 5, suborbital angle, 6, cerical groove (setae presence/absence); 7, cervical groove depth; 8, dorsolateral boss placement on carapace; 9, carapace width. Some characteristics of the great chelae showing lateral view (C) and dorsal view (D) : 1, chelae width; 2, dactyl width; 3, propodus width; 4, dactyl length; 5, dactyl and propodus opposition; 6, ventral margin ridge; 7, propodus length; 8, carpus groove; 9, carpus depth; 10, carpus length; 11, carpus dorsal tubercles; 12, tuberculation; 13, chelae width; 14, carpus width; 15, carpus dorsomesial tubercles. (E) postaxial distal part of mandible showing 8/3 dentition formula; (F) postaxial distal part of mandible showing 10/4 dentition formula; 1, corneous denticle; 2, incisor lobe; 3, molar ridge*

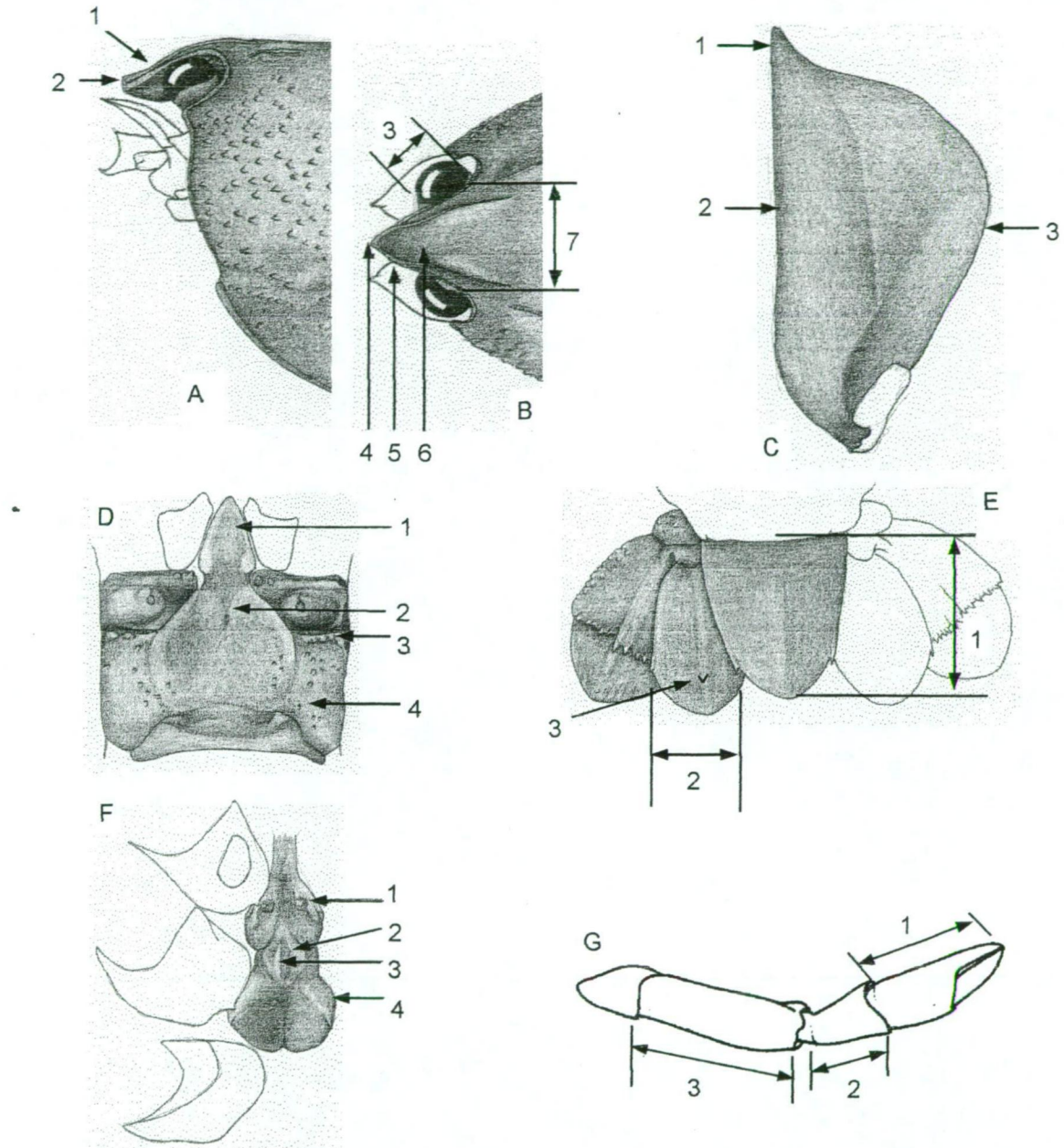


Figure 5.3. *Some characteristics of the rostrum showing lateral view (A) and dorsal view (B) : 1, lateral profile; 2, distolateral carina; 3, eye width; 4, dorsal apex; 5, dorsal carina, 6, cross-section profile; 7, rostrum width. Some characteristics of the antennal scale (C) : 1, spine; 2, lateral margin; 3, distal margin. Some characteristics of the epistome (D): 1, saggitiform anteromedian lobe; 2, posterolateral process division; 3, posterolateral margin process margin; 4, tubercle formation. Some characteristics of the tailfan (E): 1, telson length; 2, uropod endopod width; 3, uropod endopod spine. Some characteristics of the sternal keel (F) : 1, anterior lateral process; 2, mediam keel; 3, median keel mesial ridge; 4, anterior process. Some characteristics of pereopod 2 (G) : 1, chelae length; 2, carpus length; 3, merus length.*

The names of institutions where specimens are lodged have been abbreviated as follows: Australian Museum, Sydney (AM), National Museum of Victoria (NMV), School of Zoology, University of Tasmania (ZUT). The letters following the institution abbreviation are the institution code for individual specimens.

Measurement

All linear measurements of specimens were taken using Mitutoyo Vernier callipers to the nearest 0.02mm. Meristic characters of the specimens were scored viewing the specimen under a Wild M5 dissecting microscope.

Scores for each of the 66 characters were entered into Excel spreadsheets, and linear measurements were converted to ratios. Analyses of variance were performed in SYSTAT; Tukey's test was used to establish between which of the electromorphs significant differences lay. Character states were established by stem-and-leaf plot analysis in SYSTAT. Meristic and linear categories were imported into PATN; Gower Similarity coefficients were calculated between each pair of specimens, and cluster analysis was performed using UPGMA clustering. The meristic and linear categories were entered into a DELTA database (Dallwitz *et al.* 1993), from which dichotomous keys and natural language descriptions were generated.

Electromorphs were granted generic status when a species group exhibited morphological distinction combined with a substantial degree of genetic difference (Nei D >1.00). Electromorphs were granted species status where they exhibited: (1) fixed allozyme differences of at least 30% in sympatry and/or allopatry, and (2) morphological distinction in sympatry and/or allopatry. The populations within each of the species were therefore considered to share both genetic and morphological characteristics.

None of the taxonomic characters used in the descriptions were found to be sexually dimorphic, therefore all taxonomic features apply equally to males and females. In common with many other freshwater crayfish *Ombrastacoides* and *Spinastacoides* species exhibit little gross morphological variation between species, therefore few morphologically useful characters could be distinguished. Consequently it was

sometimes necessary to use a combination of characters to diagnose a species. A full key to genera and species appears in Section 5.2.

Intersex specimens were found in the following species: *O. huonensis*, *O. brevirostris* and *S. insignis*.

Illustrations

Illustrations were prepared with the aid of a Wild camera lucida mounted on a Wild M5 dissecting microscope. All species figures in the text are labelled consistently as follows;

A lateral view of carapace
B dorsal view of carapace
C lateral view of great chelae
D dorsal view of great chelae
E tailfan
F distribution map
G antennal scale
H mandible
I epistome
J sternal keel

Notes on taxonomic characters used in the keys

The majority of the taxonomic characters used in this study follow standard terminology in current literature, particularly Hobbs (187) and Horwitz (1990), however some characters require further explanation. The only readily identifiable differences between the sexes were the position and features of the openings of the gonopores on the coxae of the third (females) and fifth (males) pereopods. There does not appear to be any enlargement of the chelae in males. All taxonomic characters therefore refer to male, female and intersex adult individuals. As crayfish chelae are often subjected to loss and subsequent regeneration, differences in chelae structure have not been commented on, with the exception of the character distinguishing *S. catinipalma* from *S. inermis*. Detailed features of crayfish in these two genera are highly variable within species, for example, a character may appear in

half of the specimens of one species, but never in the specimens of another species. This feature allows species to be correctly identified at several points in the *Ombrastacoides* key B.

Rostrum – the rostrum is divided into seven characters. The width of the rostrum is measured by sliding the vernier callipers down to the eye orbit (Figure 5.4). The length measurement is explained in the character list (Table 5.2).

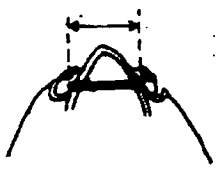


Figure 5.4. *Illustration of rostrum width measurement.*

The dorsal lateral carinae usually continue from the apex in an even, smooth arc, however in some specimens there appears to be a distinct angle, so that the carinae appear to have bowed or subparallel margins (compare the dorsal rostrum carinae on Figures 5.7B and 5.11B). The dorsal surface of the rostrum in most species is flat, however in some species it appears markedly spoon-like (when the bowl of the spoon is viewed from the top); this type of rostrum is described as "u-shaped". The reverse also occasionally occurs, where the rostrum dorsal surface appears like the bowl of a spoon viewed from the lower surface; this rostrum type is referred to as "convex".

Antennal scale – the antennal scale has four characters; these are best viewed from the ventral surface.

Mandible – The incisor lobe of the mandible of specimens from both genera typically consist of eight to ten corneous denticles. Where specimens possess eight or fewer corneous denticles, the third (when viewed from the front in-situ) is markedly larger. In specimens possessing ten corneous denticles, the first four are sub-equal, larger than the posterior denticles. The fourth denticle from the front is largest, however, the difference is not as great as in the 8/3 configuration. Many specimens

have damaged or fused denticles, and an interesting phenomena is that it appears that the right-side denticle (when viewed ventrally) is usually less likely to be damaged.

Epistome – the epistome is described as consisting of four characters. A median depression may be present only at the base of the sagittiform anteromedian lobe (Figure 5.22a), but may also extend almost to the ventral margin of the epistome (Figure 5.22b). Tubercles are present on each posterolateral section, and may be either small and clustered (Figure 5.20), or large and discreet (Figure 5.18). The distal margin of each posterolateral process may form a sharp point leading to the lateral margin (Figure 5.22a), or may consist of a continuous curve with the lateral margin (Figure 5.22b).

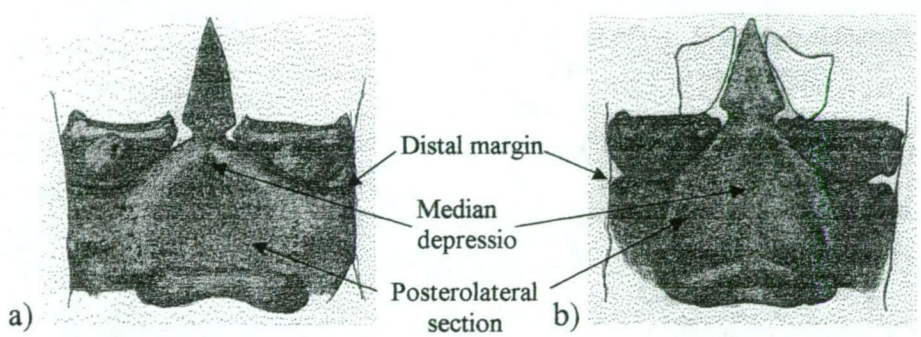


Figure 5.5. *Details of the epistome.*

Lateral cervical groove setae – these setae are long distinct setae, and not to be confused with the short stout setae projecting from each tubercle on the carapace and great chelae.

Sternal keel – The basic structure of the sternal keel is similar to other parastacids; I have concentrated on the segment between the third and fourth perepods. Figure 5.23 illustrates the basic structure and terminology used in this thesis. The sternal keel consists of 11 characters. The anterior segment of the sternal keel structure consists of a pair of alate projections termed "anterior lateral processes", with which the third pereopods articulate. The posterior segment of the sternal keel structure consists of a pair of alate projections termed "posterior lateral processes", with which the fourth pereopods articulate. A small anteromedian prominence, "the median keel", extends

between these sets of lateral processes. A distinct "mesial ridge" is apparent in some species extending centrally along the median keel.

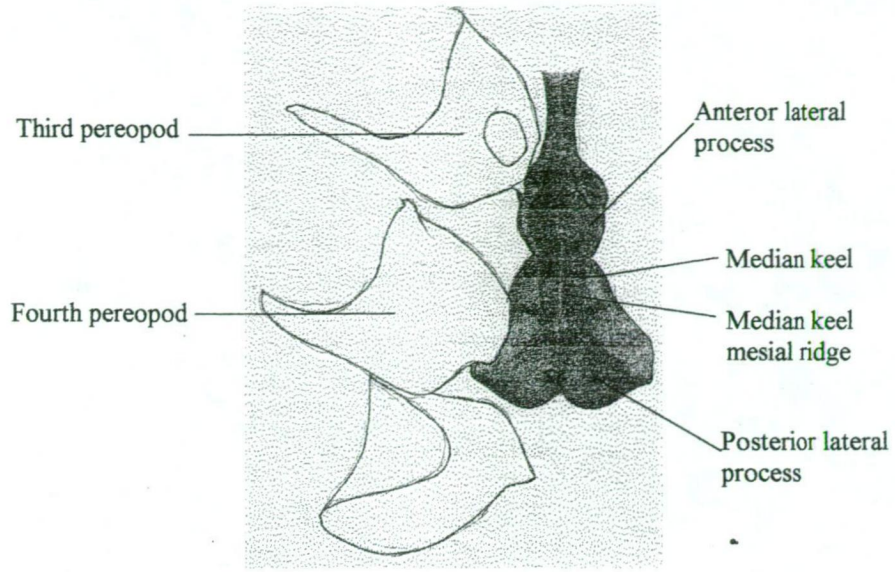


Figure 5.6. *Details of the sternal keel.*

Tailfan - The sub-terminal spine on the uropod endopod is sometimes very small, and it is best to run a pointer or similar tool up the surface of the endopod as even the smallest spines "catch" and can then be detected.

Keys

The previous chapters in this thesis have revealed the presence of two genera and 14 species in the former *Parastacoides* taxon. Four keys are provided in this section in order to enable users to identify specimens: 1) a key to identify specimens to generic level; 2) a key to identify specimens of *Spinastacoides* to species level; 3) a short key to identify *Ombrastacoides* specimens to species level and 4) a longer key to identify *Ombrastacoides* specimens to species level. These keys have been prepared on the assumption that material examined has been preserved in some way (for example, in alcohol) so colour has not been used. Characters have also been chosen which do not entail damage to the specimen, ensuring its usefulness for later analyses, or in the case of live specimens, the possibility of later release.

As with other burrowing crayfish (see Horwitz 1990), it is important to clean the specimen before analysis, as surface deposition can obscure some detail, particularly

when examining the sternal keel region. Cleaning is best accomplished by gently brushing with a small, stiff artist's brush under liquid (for example the alcohol in which the specimen has been preserved). The majority of the characters are best discerned under a dissecting microscope, however a hand-held magnifying lens is often sufficient.

Two keys have been provided for the identification of *Ombrastacoides* species; the shorter key is useful if the observer is familiar with the taxon, and all characters necessary for identification of the specimen are intact. The longer key provides characters which are easier to use by observers unfamiliar with this taxon; the longer key is also useful when key characters are damaged, as alternative or confirmatory characters are provided in some steps (where more than one character appears in a couplet, they have equal ranking and any of the characters may be used for this step of the key). *Ombrastacoides* species display a substantial degree of overlap within characters, and as a consequence, in the longer key the majority of the species can be correctly identified at several points of the key. A complete list of characters appears as Table 5.2. References to illustrations showing the character are made in brackets in the keys. These illustrations refer to the appearance of the character only; the specimen overall may differ in appearance. Brief locality notes are also given, in italics in brackets, for all species in the keys; these will assist in confirming identification.

Key to the genera *Ombrastacoides* and *Spinastacoides*

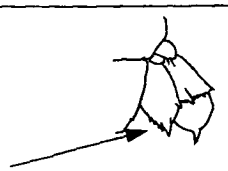
1. Terminal median spine present on uropod exopod ..... *Spinastacoides*

   Terminal median spine not present on uropod exopod ..... *Ombrastacoides*

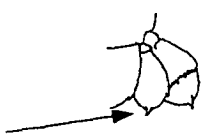
Key to the species of the genus *Spinastacoides*

1. *Uropod* endopod has a single terminal median spine and one or more mesolateral spines ..... *insignis*

   (occurs in the southwest of Tasmania, from approximately 43°S to Melaleuca in the south, and from the west coast to the upper reaches of the Huon River in the east.)

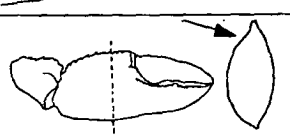


   Uropod endopod has a single terminal mesial spine 2



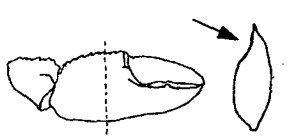
2(1). Abductor bulge of the great chelae strongly developed (see box below) ..... *inermis*

   (has a broad geographic range in southern Tasmania where it is found west of the Mt. La Perouse, Precipitous Bluff complex to the eastern shores of Bathurst Harbour, and from the eastern shores of Lake Pedder in the north to the south coast. It is also found on De Witt Island, to the south of the Tasmanian mainland)



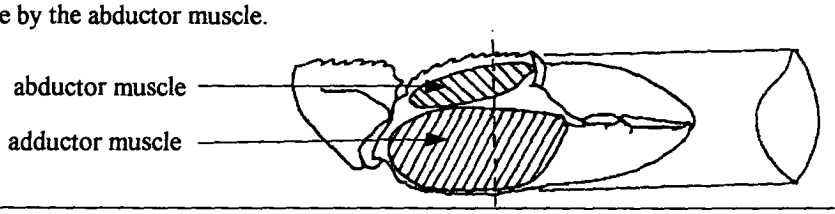
   Abductor bulge of the great chelae weakly developed, creating a distinctive, thin "dish-shaped" chelae ..... *catinipalma*

   (widely distributed in central western Tasmania, from approximately 42°S south to the northern shores of Lake Gordon, and from the west coast to the upper reaches of the River Derwent in the east)

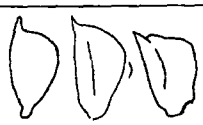
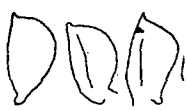
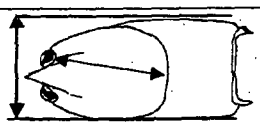
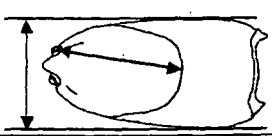
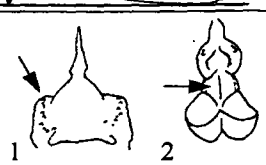
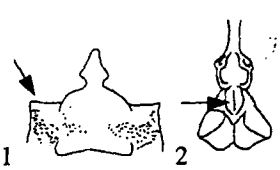


Abductor bulge

The abductor bulge is defined here as the bulge produced on the inner mesial surface of the great chelae by the abductor muscle.

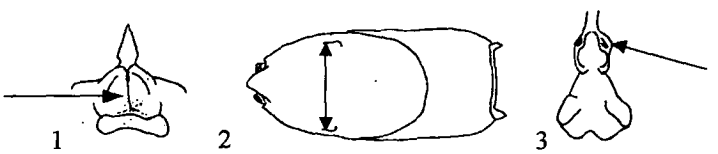


Key to the species of the genus *Ombrastacoides* (A)

1.	Rostrum profile flat in cross section ..... 2 (see Figure 5.10B)	
	Rostrum profile U-shaped in cross-section ..... 6 (see Figure 5.11B)	
	Rostrum profile convex in cross-section ..... *brevirostris* (see Figure 5.13B)	
	(widely distributed in the catchment of the Gordon River and some west coast streams)	
2(1).	Antennal scale spine well developed, sharp ..... 3 (see Figures 5.7G, 5.8G, 5.14G)	
	Antennal scale spine not well developed, often appears blunt ..... *brevirostris* (see Figures 5.9G, 5.12G)	
	(widely distributed in the catchment of the Gordon River and some west coast streams)	
3(2).	Carapace narrow (less than 50% of the OCL) ..... *parvicaudatus*	
	(extremely restricted distribution in the King River valley around the region now inundated by Lake Burbury, a hydro-electric lake, and may well be extinct as a result of this inundation)	
	Carapace wide (more than 50% of the OCL) 4	
4(3).	Latero-distal margin of epistome curved [1]; sternal keel well-rounded between pereopods 3 and 4 [2] ..... 5 (1 see Figures 5.8I, 5.9I, 5.12I, 5.14I. 2 see Figures 5.7J, 5.12J, 5.13J)	 1 2
	Latero-distal margin of epistome straight [1]; sternal keel narrow between pereopods 3 and 4 [2] ..... *asperrimanus* (1 see Figure 5.7I, 5.11I, 5.16I. 2 see Figures 5.8J, 5.9J, 5.10J)	 1 2
	(restricted range on the edges of Macquarie Harbour in the west of the State)	

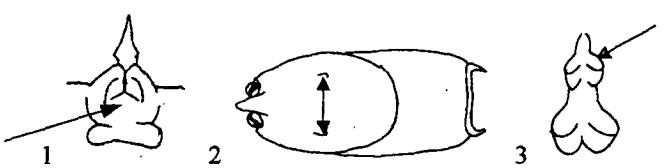
5(4). Posterolateral processes of the epistome fully divided [1]; Dorsolateral bosses placed low on carapace (distance between dorsolateral bosses/carapace width = >70% OCL) [2]; Anterior lateral processes of the sternal keel (with which the third pereopods articulate) are narrow [3] ..... *professorum*
(1 see Figures 5.7I, 5.9I, 5.11I, 5.13I. 3 see Figures 5.7J, 5.8J, 5.10J)

(restricted distribution in the King River valley near the region now inundated by Lake Burbury)



Posterolateral processes of the epistome not fully divided [1]; Dorsolateral bosses placed high on carapace (distance between dorsolateral bosses/carapace width = <70% OCL) [2]; Anterior lateral processes of the sternal keel (with which the third pereopods articulate) are broad [3] ..... *ingressus*
(1 see Figures 5.8I, 5.10I, 5.16I. 3 see Figures 5.13J, 5.15J)

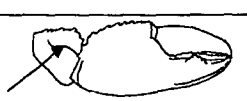
(restricted to the region around Victoria Pass in the mid west)



6(1). Mandible with third corneous denticles the largest (viewed from anterior to posterior; check right side when mandible viewed ventrally) 7
(see Figure 5.7H)

Mandible with corneous denticles 1-4 sub-equal, larger than posterior denticles (viewed from anterior to posterior; check right side when mandible viewed ventrally)
..... 11
(see Figure 5.8H)

7(6). Great chelae carpus with groove impression 8
(see Figures 5.7C, 5.8C, 5.9C)



Great chelae carpus lacking groove impression
..... *brevirostris*
(see Figure 5.10C)

(widely distributed in the catchment of the Gordon River and some west coast streams)

8(7). Dorsal carinae of rostrum straight 9
(see Figures 5.7B, 5.9B, 5.13B)

Dorsal carinae of rostrum angled 10
(see Figures 5.8B, 5.11B, 5.16B)

9(8). Dorsal apex of rostrum rounded ..... *dissitus*
(see Figures 5.9B, 5.10B, 5.12B)

(found only in the far southeast corner of the state, east of

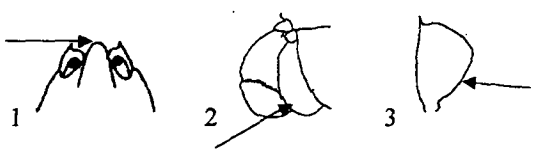
the Mt. La Perouse)

Dorsal apex of rostrum acute ..... *leptomerus*
(see Figures 5.7B, 5.8B, 5.11B)

(most widespread distribution of all Ombrastacoides *species; from approximately 42°S to the catchments of some streams and rivers flowing into Bass Strait. It is not found in the northwest or the northeast of Tasmania, and is absent from the regions west of the Inglis River catchment, and east of the Mersey River catchment.)*

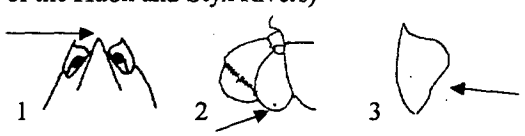
10(8). Dorsal apex of rostrum rounded [1]; Uropod endopod lacking a mesial spine [2]; Rostrum broad (RW = > RL); Mesial margin of antennal scale straight [3]; Rostrum short (RL = <10%OCL) ..... *denisoni*
(1 see Figures 5.9B, 5.10B, 5.12B. 2 see Figures 5.10E, 5.11E. 3 see Figures 5.15G, 5.15G, 5.18G)

(extremely restricted range and is known from only one population in the Little Denison River valley)

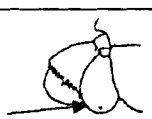


Dorsal apex of rostrum acute [1]; Uropod endopod with a mesial spine [2]; Rostrum narrow (RW = > RL); Mesial margin of antennal scale emarginate [3]; Rostrum short (RL = <10%OCL) ..... *huonensis*
(1 see Figures 5.7B, 5.8B, 5.11B. 2 see Figures 5.7E, 5.8E. 3 see Figures 5.7G, 5.10G, 5.14G)

(occurs in central-western Tasmania, around the edges of the new Lake Pedder, as well as in the upper reaches of the Huon and Styx Rivers)



11(6). Uropod endopod with sub-terminal median spine
..... *pulcher*
(see Figure5.8E)



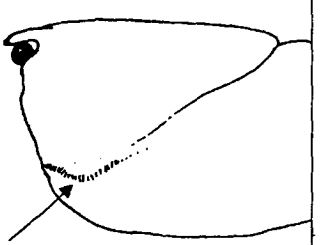
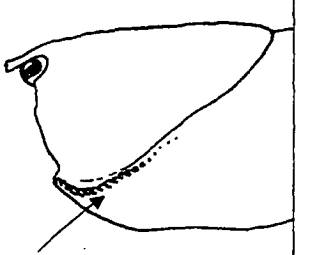
(restricted to the northern shores of Lake Pedder. The range of this species appears to have been significantly reduced by the flooding of Lake Pedder for hydro-electric power generation)

Uropod endopod lacking sub-terminal median spine
..... *decemdentatus*
(see Figure 5.11E)

(occurs in regions surrounding the Sawback and Ragged mountain ranges region of central southern Tasmania)

Key to the species of the genus *Ombrastacoides* (B)

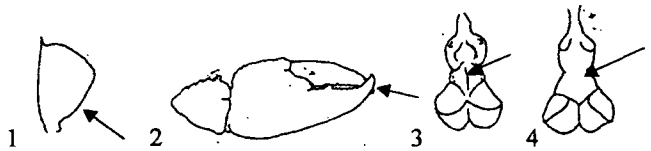
1

1.	Fewer than 8 corneous denticles in mandible, number 3 largest (viewed from anterior to posterior; check right side when mandible viewed ventrally) ..... *asperrimamus* *(restricted range on the edges of Macquarie Harbour in the west of the State)*	
	8 corneous denticles in mandible, number 3 largest (viewed from anterior to posterior; check right side when mandible when viewed ventrally) ..... 2 (see Figure 5.7H)	
	More than 8 corneous denticles in mandible (viewed from anterior to posterior; check right side when mandible when viewed ventrally) ..... 20 (see Figure 5.8H)	
2(1).	Lateral setae present on cervical groove ..... 3 (see Figure 5.10A, 5.13A, 5.18A)	
	Lateral setae absent on cervical groove (small tubercles or spines may be present) ..... 7 (see Figure 5.7A, 5.8A, 5.9A)	
3(2).	Great chelae with carpus groove impression ..... 4 (see Figures 5.7C, 5.8C, 5.9C)	
	Great chelae lacking carpus groove impression ..... *brevirostris* (see Figure 5.10C) *(widely distributed in the catchment of the Gordon River and some west coast streams)*	
4(3).	Dorsal apex of rostrum rounded ..... 5 (see Figures 5.9B, 5.10B, 5.12B)	
	Dorsal apex of rostrum acute ..... 6 (see Figures 5.7B, 5.8B, 5.11B)	

5(4). Mesial margin of antennal scale straight [1]; Great chelae dactyl and propodus crossing distally [2]; Median ridge of sternal keel well-developed [3]; Posterior process of sternal keel narrow [4] ..... *denisoni*

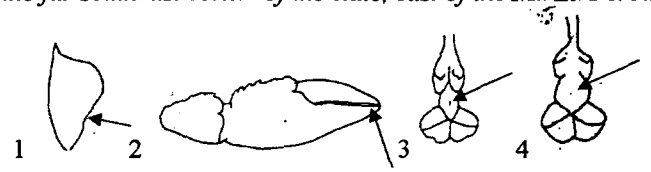
(1 see Figures 5.15G, 5.15G, 5.18G. 2 see Figure 5.12C, 5.14C. 3 see Figures 5.9J, 5.10J, 5.11J. 4 see Figures 5.10J, 5.11J, 5.13J)

(extremely restricted range and is known from only one population in the Little Denison River valley)



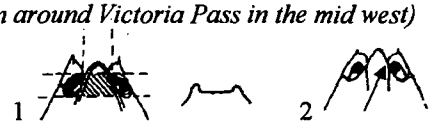
Distal margin of antennal scale emarginate [1]; Great chelae dactyl and propodus meeting distally [2]; Median ridge of sternal keel not well-developed [3]; Posterior process of sternal keel broad [4] ..... *dissitus*
(1 see Figures 5.7G, 5.10G, 5.14G. 2 see Figures 5.7C, 5.8C, 5.11C. 3 see Figures 5.7J, 5.8J, 5.12J. 4 see Figures 5.9J, 5.12J, 5.14J)

(found only in the far southeast corner of the state, east of the Mt. La Perouse)



6(4). Rostrum profile flat in cross section [1]; Dorsal carinae of rostrum angled [2] ..... *ingressus*
(1 see Figure 5.10B. 2 see Figures 5.8B, 5.11B, 5.16B)

(restricted to the region around Victoria Pass in the mid west)



Rostrum profile U-shaped in cross section [1]; Dorsal carinae of rostrum straight [2] ..... *leptomerus*
(1 see Figure 5.11B, 2 see Figures 5.7B, 5.9B, 5.13B)

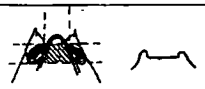
(most widespread distribution of all Ombrastacoides *species; from approximately 42°S to the catchments of some streams and rivers flowing into Bass Strait. It is not found in the northwest or the northeast of Tasmania, and is absent from the regions west of the Inglis River catchment, and east of the Mersey River catchment.)*

7(2). Great chelae carpus with groove impression 8
(see Figures 5.7C, 5.8C, 5.9C)

Great chelae carpus lacking groove impression 19
(see Figure 5.10C)

8(7). Rostrum broad (width greater than length) 9
Rostrum narrow(length greater than width) 14

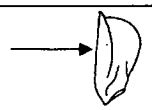
9(8). Rostrum profile flat in cross section 10
(see Figure 5.10B)



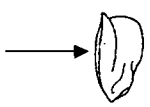
Rostrum profile U-shaped in cross-section .... *leptomerus*
(see Figure 5.11B)

(most widespread distribution of all Ombrastacoides *species; from approximately 42°S to the catchments of some streams and rivers flowing into Bass Strait. It is not found in the northwest or the northeast of Tasmania, and is absent from the regions west of the Inglis River catchment, and east of the Mersey River catchment.)*

10(9). Spine of antennal scale forming lateral margin 11
(see Figures 5.7G, 5.8G, 5.9G)

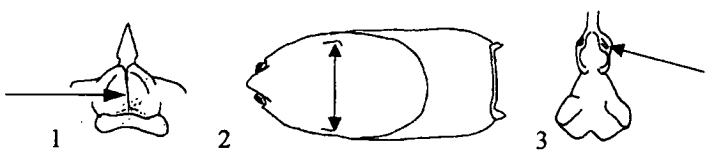


Spine of antennal scale not forming lateral margin 12
(see Figures 5.18G, 5.20G)



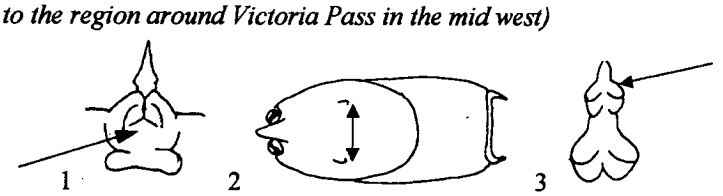
11(10). Posterolateral processes of the epistome fully divided [1]; Dorsolateral bosses placed low on carapace (distance between dorsolateral bosses/carapace width = >70% OCL) [2]; Anterior lateral processes of the sternal keel (with which the third pereopods articulate) are narrow [3] ..... *professorum*
(1 see Figures5.7I, 5.9I, 5.11I. 3 see Figures 5.7J, 5.8J5.10J)

(restricted distribution in the King River valley near the region now inundated by Lake Burbury)

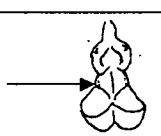


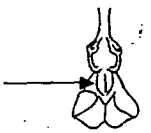
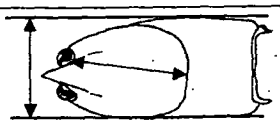
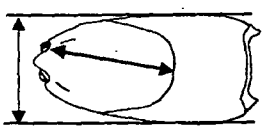
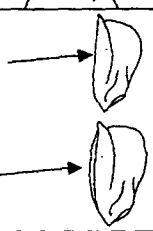
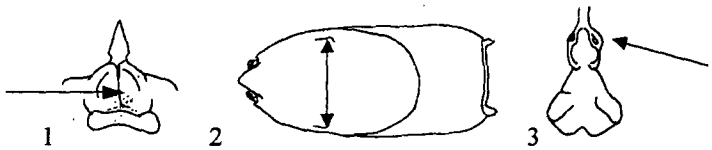
Posterolateral processes of the epistome not fully divided [1]; Dorsolateral bosses placed high on carapace (distance between dorsolateral bosses/carapace width = <70% OCL) [2]; Anterior lateral processes of the sternal keel (with which the third pereopods articulate) are broad [3] ..... *ingressus*
(1 see Figures 5.8I, 5.10I, 5.16I. 3 see Figures 5.13J, 5.15J)

(restricted to the region around Victoria Pass in the mid west)



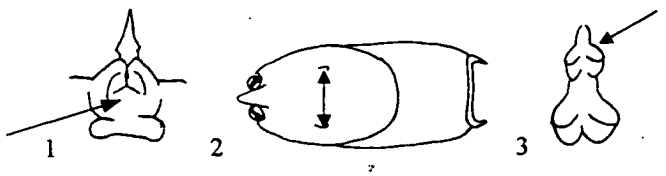
12(10). sternal keel well-rounded between pereopods 3 and 4
..... 13
(see Figures 5.7J, 5.12J, 5.13J)



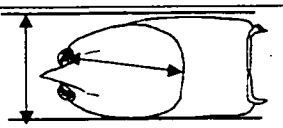
	sternal keel narrow between pereopods 3 and 4 ..... *asperrimanus* (see Figures 5.8J,5.9J, 5.10J) *(restricted range on the edges of Macquarie Harbour in the west of the State)*	
13(12).	Carapace narrow (less than 50% of the OCL) ..... *parvicaudatus* *(extremely restricted distribution in the King River valley around the region now inundated by Lake Burbury, a hydro-electric lake, and may well be extinct as a result of this inundation)* Carapace wide (more than 50% of the OCL) ..... *ingressus* *(restricted to the region around Victoria Pass in the mid west)*	 
14(8).	Rostrum profile flat in cross section ..... 15 (see Figure 5.10B) Rostrum profile U-shaped in cross-section .... 18 (see Figure 5.11B)	
15(14).	Spine of antennal scale forming lateral margin ..... 16 (see Figures 5.7G, 5.8G, 5.9G) Spine of antennal scale not forming lateral margin ..... 17 (see Figures 5.18G, 5.20G)	
16(15).	Posterolateral processes of the epistome fully divided [1]; Dorsolateral bosses placed low on carapace (distance between dorsolateral bosses/carapace width = >70% OCL) [2]; Anterior lateral processes of the sternal keel (with which the third pereopods articulate) are narrow [3] ... . *professorum* (1 see Figures5.7I, 5.9I, 5.11I. 3 see Figures 5.7J, 5.8J5.10J) *(restricted distribution in the King River valley near the region now inundated by Lake Burbury)* 1 2 3	

Posterolateral processes of the epistome not fully divided [1]; Dorsolateral bosses placed high on carapace (distance between dorsolateral bosses/carapace width = <70% OCL) [2]; Anterior lateral processes of the sternal keel (with which the third pereopods articulate) are broad [3] ..... *ingressus*
(1 see Figures 5.8I, 5.10I, 5.16I. 3 see Figures 5.13J, 5.15J)

(restricted to the region around Victoria Pass in the mid west)

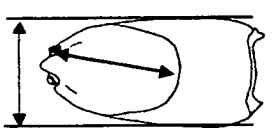


17(15). Carapace narrow (less than 50% of the OCL) ..... *parvicaudatus*



(extremely restricted distribution in the King River valley around the region now imundated by Lake Burbury, a hydro-electric lake, and may well be extinct as a result of this imundation)

Carapace wide (more than 50% of the OCL) ..... *ingressus*



(restricted to the region around Victoria Pass in the mid west)

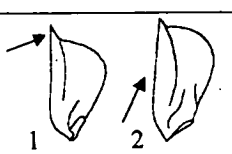
18(14). Dorsal carinae of rostrum straight ..... *leptomerus*
(see Figures 5.7B, 5.9B, 5.13B)

(most widespread distribution of all Ombrastacoides *species; from approximately 42°S to the catchments of some streams and rivers flowing into Bass Strait. It is not found in the northwest or the northeast of Tasmania, and is absent from the regions west of the Inglis River catchment, and east of the Mersey River catchment.)*

Dorsal carinae of rostrum angled ..... *huonensis*
(see Figures 5.8B, 5.11B, 5.16B)

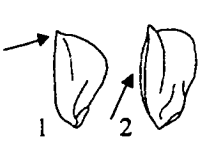
(occurs in central-western Tasmania, around the edges of the new Lake Pedder, as well as in the upper reaches of the Huon and Styx Rivers)

19(7). Antennal scale spine well developed, sharp [1]; Spine of antennal scale forming lateral margin [2] ..... *professorum*
(1 see Figures 5.7G, 5.8G, 5.14G. 2 see Figures 5.7G, 5.8G, 5.9G)



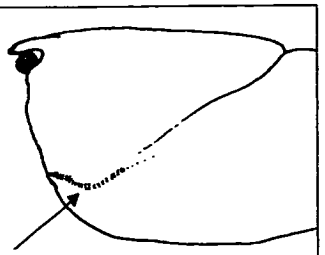
(restricted distribution in the King River valley near the region now imundated by Lake Burbury)

Antennal scale spine strength weak [1]; Spine of antennal scale not forming lateral margin [2] ..... *brevirostris*
(1 see Figures 5.9G, 5.12G. 2 see Figures 5.18G, 5.20G)

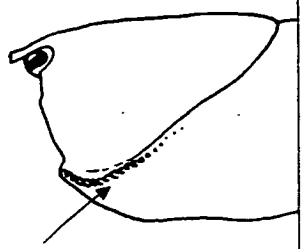


(widely distributed in the catchment of the Gordon River and some west coast streams)

20(1). Lateral setae present on cervical groove 21
(see Figure 5.10A, 5.13A, 5.18A)



Lateral setae absent on cervical groove (small tubercles or spines may be present) 26
(see Figure 5.7A, 5.8A, 5.9A)



21(20). Great chelae carpus with groove impression 22
(see Figures 5.7C, 5.8C, 5.9C)

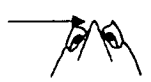
Great chelae carpus lacking groove impression ..... *brevirostris*
(see Figure 5.10C)

(widely distributed in the catchment of the Gordon River and some west coast streams)

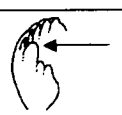
22(21). Dorsal apex of rostrum rounded ..... *dissitus*
(see Figures 5.9B, 5.10B, 5.12B)

(found only in the far southeast corner of the state, east of the Mt. La Perouse)

Dorsal apex of rostrum acute 23
(see Figures 5.7B, 5.8B, 5.11B)



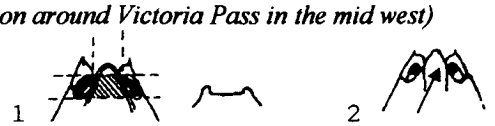
23(22). Mandible with third corneous denticles the largest (viewed from anterior to posterior; check right side when mandible viewed ventrally) 24
(see Figure 5.7H)



Mandible with corneous denticles 1-4 sub-equal, larger than posterior denticles (viewed from anterior to posterior; check right side when mandible when viewed ventrally) 25
(see Figure 5.8H)

24(23). Rostrum profile flat in cross section [1]; Dorsal carinae of rostrum angled [2] ..... *ingressus*
(1 see Figure 5.10B. 2 see Figures 5.8B, 5.11B, 5.16B)

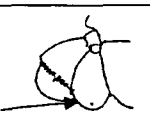
(restricted to the region around Victoria Pass in the mid west)



Rostrum profile U-shaped in cross section [1]; Dorsal carinae of rostrum straight [2]
..... *leptomerus*
(1 see Figure 5.11B, 2 see Figures 5.7B, 5.9B, 5.13B)

(most widespread distribution of all Ombrastacoides *species; from approximately 42°S to the catchments of some streams and rivers flowing into Bass Strait. It is not found in the northwest or the northeast of Tasmania, and is absent from the regions west of the Inglis River catchment, and east of the Mersey River catchment.)*

25(23). Uropod endopod with sub-terminal median spine
..... *pulcher*
(see Figure5.8E)



(restricted to the northern shores of Lake Pedder. The range of this species appears to have been significantly reduced by the flooding of Lake Pedder for hydro-electric power generation)

Uropod endopod lacking sub-terminal meadian spine
..... *decemdentatus*
(see Figure 5.11E)

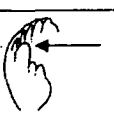
(occurs in regions surrounding the Sawback and Ragged mountain ranges region of central southern Tasmania)

26(20). Great chelae carpus with groove impression 27
(see Figures 5.7C, 5.8C, 5.9C)

Great chelae carpus lacking groove impression
.....*brevirostris*
(see Figure 5.10C)

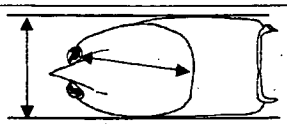
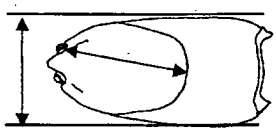
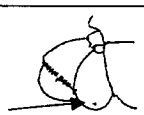
(widely distributed in the catchment of the Gordon River and some west coast streams

27(26). Mandible with third corneous denticles the largest (viewed from anterior to posterior; check right side when mandible viewed ventrally) 28
(see Figure 5.7H)



Mandible with corneous denticles 1-4 sub-equal, larger than posterior denticles (viewed from anterior to posterior; check right side when mandible when viewed ventrally)
..... 30
(see Figure 5.8H)

28(27). Dorsal carinae of rostrum straight 29
(see Figures 5.7B, 5.9B, 5.13B)

	Dorsal carinae of rostrum angled ..... *leptomerus* (see Figures 5.8B, 5.11B, 5.16B) *(most widespread distribution of all* Ombrastacoides *species; from approximately 42°S to the catchments of some streams and rivers flowing into Bass Strait. It is not found in the northwest or the northeast of Tasmania, and is absent from the regions west of the Inglis River catchment, and east of the Mersey River catchment.)*	
29(28).	Carapace narrow (less than 50% of the OCL) ..... *parvicaudatus* *(extremely restricted distribution in the King River valley around the region now inundated by Lake Burbury, a hydro-electric lake, and may well be extinct as a result of this inundation)* Carapace wide (more than 50% of the OCL) ..... *ingressus* *(restricted to the region around Victoria Pass in the mid west)*	 
30(27).	Uropod endopod with sub-terminal median spine ..... *pulcher* (see Figure5.8E) *(restricted to the northern shores of Lake Pedder. The range of this species appears to have been significantly reduced by the flooding of Lake Pedder for hydro-electric power generation)* Uropod endopod lacking sub-terminal meadian spine ..... *decemdentatus* (see Figure 5.11E) *(occurs in regions surrounding the Sawback and Ragged mountain ranges region of central southern Tasmania)*	 

Systematics

Species Descriptions

Previously described species are listed in chronological order; new species are listed in alphabetical order.

Order DECAPODA

Infraorder ASTACIDEA

Superfamily PARASTACIDEA

Family PARASTACIDAE

Genus *Ombrastacoides*, gen. nov.

Etymology: Ombrastacoides; compound noun; "rain crayfish", from *ombros*, (Greek) rain and *astacoides*, (Latin) crayfish-like, referring to its distribution in areas receiving more than 1000mm rain per annum. Gender: masculine.

Type species: *Allotype Male* (AMP11980) 17.55 mm OCL, in outlet stream, Lake Lilla, Cradle Mountain, Tas., 01.02.1949. E.F. Riek.

Description

Total length rarely more than 80mm. Antennae at least length of carapace, inner flagellum of antennule shorter than outer flagellum. *Antennal scale* lateral margin straight to curved; spine weak to strong, usually producing from lateral margin; distal margin entire, excavate, or curved. *Rostrum* short and wide to long and narrow; rostral cross-section profile straight, concave, or convex; rostral dorsal carina margin straight or angled, apex rounded to acute; rostral lateral profile anteriorly depressed, straight, or upturned, rostrum distally acute. *Eye* variable in size; eye orbit posterior margin notched or entire; suborbital angle deeply curved to truncate. *Mandible* bearing 7-10 corneous denticles, numbers 3 or 4 largest, dentition formula usually 8-3 or 10-4. *Epistome* sagittiform, anteromedian lobe short and wide to long and narrow; posterolateral processes either partially or fully divided, tubercles on processes ranging from small and clustered to large and discrete, distal margins curved to straight.

Cephalothorax and *Carapace* variable in length, width and depth, setose, tuberculate or both, anteroventral cephalon more coarsely ornamented than branchiostegites; position of dorsolateral bosses variable both in distance from eye orbit, and in relation to carapace width; cervical groove impression shallow to very deep, dorsally with deeply rounded U-shape (sometimes notched); cervical groove lateral setae sometimes present. Anterolateral extension of branchiocardiac grooves distinct but close to cervical groove. Areola broad. Cervical and branchiocardiac grooves close, but obviously separated.

Great chelae variable in length (but approximate with OCL), width and depth; often with distinctive ventral ridge; chelae lateral propodal surface tuberculate to setose-tuberculate; adductor boss development weak to strong. Dactyl and propodus variable in length and depth; dactyl and propodus meeting, crossing, overlapping, or crossing and overlapping. Carpus variable in length, width and depth; 3 to 7 dorsal tubercles usually forming distinct row; dorsomesial tubercle row often present; carpal groove impression weak to strong, occasionally absent. *Pereopod 2* variable in length (but approximate with OCL).

Sternal keel anterior lateral process shallow to deep, distally pointed to rounded; anterior margins of processes shorter, equal to, or longer than posterior margins; processes often meeting centrally. Median keel narrow to well-rounded; median keel mesial ridge weak to strong. Posterior process shallow to deep, narrow to broad; anterior margins of processes straight to curved, shorter, or equal to, rarely longer than posterior margins; processes often meeting centrally. Male genitalia consisting of a large, nonlobed, fleshy, semi-cylindrical outgrowth on mesial side of coxa.

Uropod endopod variable; mesial non-terminal spine either single non-terminal or absent.

Brachial formula 17+epr; pleurobranchiae absent; posterior arthrobranchiae reduced; stem of podobranchiae without winglike expansions.

Remarks

Specimens rarely intersexed (with both male and female gonopores).

***Ombrastacoides leptomerus* Riek, 1951**

(Figure 5.7)

Parastacoides leptomerus Riek, 1951: 387.

Parastacoides setosimerus Riek, 1951

Parastacoides setosimerus Riek, 1951: 386

Parastacoides tasmanicus tasmanicus Sumner, 1978:819

LT, Hansen and Richardson 1999a

Etymology: leptomerus; Riek does not provide the etymology of this name, however; Greek compound noun; from *lepto*, slender and *meros*, the proximal segment of the hind limb.

Material examined

Allotype Male (AMP11980) 17.55 mm OCL, in outlet stream, Lake Lilla, Cradle Mountain, Tas., 01.02.1949. E.F. Riek.

Paratype Male (AMP11981) 18.14 mm OCL, in outlet stream, Lake Lilla, Cradle Mountain, Tas., E.F.Riek.

Other material examined.

P. setosimerus Holotype Male (P11976) 16.62 mm OCL, Mt Rufus 4000 feet, 25.1.1949, E. F. Riek.

P. setosimerus Allotype Female (P11977) 31.66 mm OCL, Mt Rufus 1219 metres, 25.1.1949, E. F. Riek.

♂ (ZUT IRT9) 19.02 mm OCL, under rocks in creek at tributary of Inglis River on Choveaux Road near Takone, Tas., 8015: 858 389, 12.02.1986, PH, AF. ♂ (ZUT IRT10) 22.62 mm OCL, same data as IRT9 except 28.11.1985, PH, PHu,. ♂ (ZUT IRT18) 16.72 mm OCL, same data as IRT9 except 9.02.1988, PH, AMMR, RBM. ♂ (ZUT NCF5) 17.24 mm OCL, in deep complex burrow in wet peat under buttongrass and Melaleuca heath at Newton Creek valley Tyndall Range, Tas., 8014: 821 598, 2.05.1988, AMMR, RBM, RH, PH. ♂ (ZUT NSC10) 22.74 mm OCL, in shallow burrow to rock under short heath, some dry, several dead animals, sympatric with *Engaeus*, at Newton Creek valley Tyndall Range, Tas., 8014: 821 598, 2.05.1988,

AMMR, RBM, RH, PH. ♂ (ZUT NSC 11) 20.66 mm OCL, same data as NSC10. ♂ (ZUT RT10) 24.26 mm OCL, under rocks in creek at Rubbish Tip Creek, Penguin, Tas., 8115, 212 451, 11.11.1983, AMMR, RS. ♂ (ZUT RT11) 16.48 mm OCL, same data as RT10 except AMMR, RS, DS. ♂ (ZUT RT15) 18.04 mm OCL, same data as RT10. ♀ (ZUT IRT11) 20.16 mm OCL, same data as IRT9. ♀ (ZUT IRT12) 21.00 mm OCL, same data as IRT10. ♀ (ZUT IRT13) 25.42 mm OCL, same data as IRT10. ♀ (ZUT NSC7) 24.56 mm OCL, same data as NSC10. ♀ (ZUT NSC8) 26.16 mm OCL, same data as NSC10. ♀ (ZUT NCF9) 27.00 mm OCL, same data as NCF5. ♀ (ZUT RT12) 20.18 mm OCL, same data as RT11. ♀ (ZUT RT13) 19.58 mm OCL, same data as RT10. ♀ (ZUT RT14) 24.10 mm OCL, same data as RT10.

Diagnosis

Rostrum profile concave in cross-section, dorsal carina margin straight, apex acute; mandible corneous denticle #3 the largest; non-terminal spine on uropod exopod; antennal scale spine strong.

Description:

Antennal scale lateral margin usually straight, sometimes curved; spine strong, forming lateral margin; distal margin usually entire, sometimes excavate. *Rostrum* length 0.1-0.13 OCL, width 0.73-0.99 RL; rostral profile concave in cross-section; rostral dorsal carina margin straight, apex acute; rostral lateral profile straight or anteriorly depressed, margin distolaterally blunt to acute. *Eye* 0.06-0.09 OCL; posterior margin of orbit entire; suborbital angle usually truncate, rarely curved. *Mandible* dentition formula usually 8-3, occasionally 10-3. *Epistome* sagittiform, anteromedian lobe short, wide; posterolateral processes usually partially divided, tubercles small, clustered to large, discrete, distal margin usually curved.

Cephalothorax length 0.71-0.83 OCL. *Carapace* width 0.48-0.53 OCL, depth 0.55-0.62 OCL; dorsolateral bosses 0.31-0.38 OCL in distance from eye orbit, position on carapace 0.66-0.74 CW; cervical groove deep to very deep, deeply rounded U in dorsal view, occasionally notched, lateral setae usually absent.

Great chelae length 0.82-1.12 OCL, width 0.42-0.49 chelae length, depth 0.62-0.68 chelae width; chelae ventral ridge rarely extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss usually well-developed. Dactyl length 0.47-0.55 chelae length, depth 0.23-0.37 dactyl length; propodus length 0.34-0.41 chelae length, depth 0.48-0.66 propodus length; dactyl and propodus usually meeting, occasionally overlapping, distally. Carpus length 1.04-1.39 carpus width, width 0.55-0.76 carpus length, depth 0.72-0.96 carpus width; 4-6 dorsal tubercles forming distinct row; dorsomesial tubercle row absent; carpus groove weak to well-developed. *Pereopod 2* length 0.81-0.95 OCL, chelae 0.34-0.38 pereopod length.

Sternal keel anterior lateral process usually deep, distally pointed; anterior margins of processes usually shorter than, or equal to, posterior margins; processes usually meeting centrally. Median keel narrow to well-rounded; mesial ridge weakly-developed to well-developed. Posterior process usually deep and broad; anterior margins of processes usually curved, anterior margins of processes usually shorter than, or equal to, posterior margins, processes usually meeting centrally.

Uropod endopodite bearing single, non-terminal spine; endopod width 0.64-0.73 endopod length; telson length 0.34-0.41 OCL.

Allotype male

Antennal scale lateral margin straight; spine intermediate in strength, produced from lateral margin; distal margin entire. *Rostrum* length 0.13 OCL, width 0.83 rostrum length; rostral profile concave in cross-section; rostral dorsal carina margin straight, apex acute; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.08 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe short, wide; posterolateral processes partially divided, tubercles large, discrete, distal margin straight.

Cephalothorax length 0.65 OCL. *Carapace* width 0.51 OCL, depth 0.56 OCL; dorsolateral bosses 0.31 OCL from eye orbit, lateral position 0.61 carapace width; cervical groove deep, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.91 OCL, width 0.49 chelae length, depth 0.67 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.5 chelae length, depth 0.35 dactyl length; propodus length 0.36 chelae length, depth 0.53 propodus length; dactyl and propodus overlapping distally. Carpus length 1.20 carpus width, width 1.30 carpus length, depth 0.83 carpus length; 5 dorsal tubercles forming distinct row; dorsomesial tubercle row absent; carpus groove well-developed. *Pereopod 2* length 0.99 OCL, chelae 0.38 pereopod length.

Sternal keel anterior lateral process shallow, distally rounded; anterior and posterior margins of processes equal; processes meeting centrally. Median keel narrow; mesial ridge well-developed. Posterior process shallow, narrow; anterior margins of processes straight, anterior and posterior margins of processes equal, processes meeting centrally.

Uropod endopodite bearing single, non-terminal spine; endopod width 0.68 endopod length; telson 0.37 OCL.

Paratype male

Specimen as per Allotype except: *Antennal scale* spine not produced from lateral margin. *Epistome* posterolateral processes distal margin curved. *Great chelae* adductor boss strongly developed; dactyl and propodus directly opposed distally, tips not overlapping.

Morphological Variation

The extensive range of *O. leptomerus* explains much of the morphological variation observed, however intrapopulational variability is also evident. As with other

widespread *Ombrastacoides* species, the population may be morphologically distinct at some localities in its range.

The antennal scale from populations at Newton Creek and Inglis River (NSC and IRT above) varies from the Allotype; the spine is not produced from the lateral margin. The distal margin is excavate in the Rubbish Tip Creek population (RT). The rostral carinae are typically distolaterally blunt, but acute at Inglis River.

The arrangement of mandibular denticles generally conforms with the Allotype, but three individuals possessed a mandible bearing more than eight corneous denticles, however, the third corneous denticle was always largest.

The posterolateral processes of the epistome bear large and discrete tubercles in the Newton Creek population.

The carapace is usually stocky; but there is a tendency in specimens from Rubbish Tip Creek for it to be longer. Newton Creek specimens have a more deeply expressed cervical groove, while specimens from Rubbish Tip Creek usually show a dorsal notch in the cervical groove.

Little variation is evident in the shape of the great chelae, although there is a tendency for it to be narrower than in the Allotype. However, the adductor boss is usually strongly developed, and the ventral ridge does occasionally extend proximal to the propodus cutting surface. In many populations the dactyl and propodus meet distally, but in the Rubbish Tip Creek population the dactyl and propodus cross distally. The carpus is more variable in shape than the chelae; those from Inglis River are more robust. Dorsal tubercles on the carpus vary from four to six in number, but the usual condition is for four. A carpal groove is always present, but the strength of expression is variable.

Although the median keel of the sternal keel is usually well-rounded, the mesial ridge is often not well-developed (particularly in Rubbish Tip Creek population).

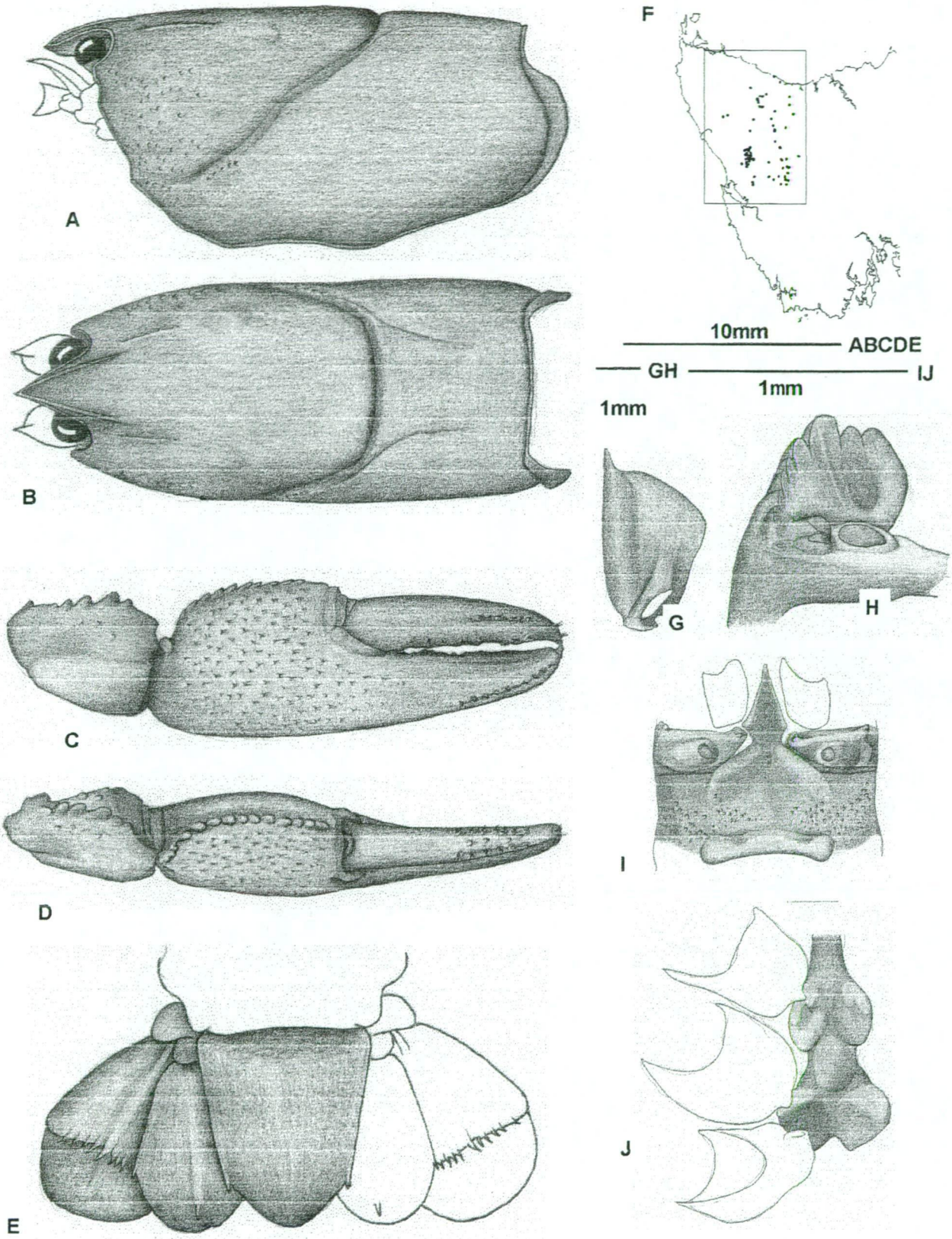


Figure 5.7. Ombrastacoides leptomerus; *A-J from IRT18.*

***Ombrastacoides pulcher* Reik, 1967**

(Figure 5.8)

Parastacoides pulcher Riek, 1967:1006, Sumner, 1978:810, Crandall *et al.*, 1995: 22

Parastacoides tasmanicus tasmanicus Sumner, 1978:819

SPTB, Hansen and Richardson 1999a

Etymology: Riek does not provide the etymology of this name, however; Latin adjective; *pulcher,* beautiful, handsome or noble.

Material examined

Holotype Male (NMVJ908) 15.28 mm OCL, Lake Pedder, SW Tas., 01.02.1965, A. Neboiss.

Other material examined

♂ (ZUT V1208) 20.00 mm OCL, east of dune system, under reed clumps at north bank of Maria Creek, Lake Pedder, Tas., 8112: 374 466, 21.02.1972, BK, TT. ♂ (ZUT 1210) 12.40 mm OCL, same data as V1208. ♂ (ZUT V1218) 21.78 mm OCL, in swamp in south-west corner of Lake Pedder, under Citadels, 8112: 270 442, 21.02.1972, BK, TT. ♂ (ZUT V1219) 18.32 mm OCL, same data as V1218. ♂ (ZUT V1221) 16.24 mm OCL, same data as V1218. ♀ (ZUT V1206) 13.88 mm OCL, same data as V1208. ♀ (ZUT V1207) 13.20 mm OCL, same data as V1208. ♀ (ZUT V1209) 14.82 mm OCL, same data as V1208. ♀ (ZUT V1214) 23.04 mm OCL, same data as V1218. ♀ (ZUT V1216) 14.02 mm OCL, same data as V1218. ♀ (ZUT V1222) 22.36 mm OCL, same data as V1218. IS (ZUT V1204) 20.52 mm OCL, same data as V1208.

Diagnosis

Mandible dentition formula 10-4; uropod endopod bearing non-terminal mesial spine.

Description:

Antennal scale lateral margin straight; spine strong, produced from lateral margin; distal margin entire. *Rostrum* length 0.13-0.2 OCL, width 0.66-0.87 rostrum length; rostral profile straight in cross-section; rostral dorsal carina margin straight or angled, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.01-0.13 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 10-4. *Epistome* sagittiform, anteromedian lobe short and wide or long and narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.65-0.86 OCL. *Carapace* width 0.54-0.6 OCL, depth 0.62-0.68 OCL; dorsolateral bosses 0.26-0.36 OCL from eye orbit, lateral position 0.53-0.64 carapace width; cervical groove shallow, deeply rounded U in dorsal view, lateral setae usually absent.

Great chelae length 0.85-1.11 OCL, width 0.35-0.47 chelae length, depth 0.55-0.65 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface tuberculate or setose-tuberculate; chelae adductor boss usually strongly developed. Dactyl length 0.54-0.6 chelae length, depth 0.2-0.32 dactyl length; propodus length 0.38-0.49 chelae length, depth 0.4-0.6 chelae length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.06-1.35 carpus width, depth 0.74-0.94 carpus length, width 1.20-1.36 carpus depth; up to 3 dorsal tubercles usually forming distinct row; dorsomesial tubercle row usually present; carpus groove well-developed. *Pereopod 2* length 0.67-0.9 OCL, chelae 0.36-0.46 pereopod length.

Sternal keel anterior lateral process usually shallow, distally pointed; anterior margins of processes longer than posterior margins; processes not meeting centrally. Median keel intermediate to well-rounded; mesial ridge often well-developed. Posterior process deep, broad; anterior margins of processes curved, shorter than posterior margins, processes meeting centrally.

Uropod endopodite bearing single, non-terminal spine; endopod width 0.6-0.76 endopod length; telson length 0.37-0.46 OCL.

Holotype Male

Antennal scale lateral margin straight; spine strong, produced from lateral margin; distal margin entire. *Rostrum* length 0.17 OCL, width 0.9 rostrum length; rostral profile straight in cross-section; rostral dorsal carina margin straight, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.11 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 10-4. *Epistome* sagittiform, anteromedian lobe short, wide; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.65 OCL. *Carapace* width 0.53 OCL, depth 0.65 OCL; dorsolateral bosses 0.33 OCL from eye orbit, lateral position 0.65 carapace width; cervical groove shallow, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.9 OCL, width 0.51 chelae length, depth 0.59 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.55 chelae length, depth 0.39 dactyl length; propodus length 0.39 chelae length, depth 0.75 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.40 carpus width, depth 0.71 carpus length, width 0.99 carpus depth; 3 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpus groove well-developed. *Pereopod 2* length 0.98 OCL, chelae 0.37 pereopod length.

Sternal keel anterior lateral process deep, distally pointed; anterior margins of processes longer than posterior margins; processes not meeting centrally. Median keel intermediate in width; mesial ridge well-developed. Posterior process deep, broad; anterior margins of processes curved, shorter than posterior margins, processes meeting centrally.

Uropod endopodite bearing single, non-terminal spine; endopod width 0.71 endopod length; telson length 0.39 OCL.

Morphological Variation

Some morphological variability is expressed by specimens of *O. pulcher*, but certain characters are stable and diagnostic. The mandible always bears ten corneous denticles, the fourth of which is the largest. *O. pulcher* is readily distinguishable from *O. decemdentatus* (see below), which also exhibits this mandibular configuration, by the presence of a non-terminal mesial spine on the endopodite of the uropod.

Typically, the antennal scale spine is not produced from the lateral margin as seen in the Holotype, while the distal margin is usually curved. The carapace of the swamp specimens (ZUT V1218,19,21,22) conforms in shape to the Holotype, but Maria Creek specimens tend towards a slightly narrower, shallower carapace.

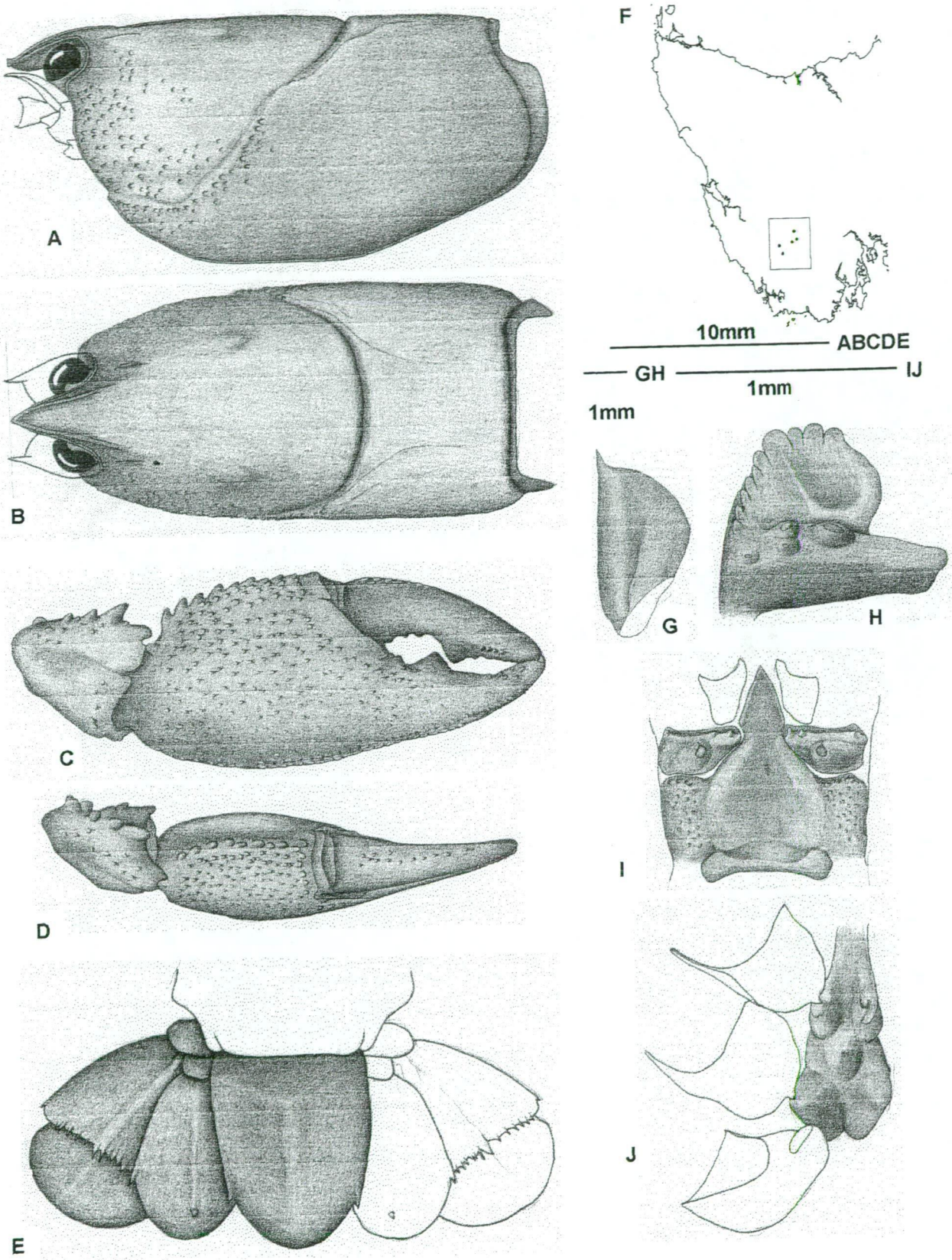


Figure 5.8. Ombrastacoides pulcher. *A-J from V1218.*

***Ombrastacoides asperrimanus*, sp. nov.**

(Figure 5.9)

RCT, Hansen and Richardson 1999a

Etymology: asperrimanus Latin compound noun: "rough claw". From *asper*, rough and *manus*, hand; referring to the strong tuberculation on the lateral surface of the propodus of the great chelae.

Material examined

Holotype Male (ZUT BIR9) 22.62 mm OCL, shallow burrow under moss and tussock in seepage down track, sympatric with WCT and *Engaeus*, at Birchs Inlet, Landing Creek quarry, Tas., 7912: 736 926, 1.05.1988, AMMR, RBM, RH, PH.
Allotype Female (ZUT BIR18) 25.82 mm OCL, same data as Holotype.
Paratypes. ♂ (ZUT BIR13) 20.56 mm OCL, same data as Holotype. ♂ (ZUT BIR22) 24.46 mm OCL, same data as Holotype. ♀ (ZUT BIR14) 27.62 mm OCL, same data as Holotype. ♀ (ZUT BIR19) 29.36 mm OCL, same data as Holotype.

Diagnosis

Sternal keel median keel narrow; rostrum long, broad; cervical groove lateral setae absent.

***Description*:**

Antennal scale lateral margin straight; spine intermediate in strength, not produced from lateral margin; distal margin entire. *Rostrum* length 0.1-0.12 OCL, width 1-1.2 rostrum length; rostral profile straight in cross-section; rostral dorsal carina margin usually straight, apex acute; rostral lateral profile straight or anteriorly depressed, margin distolaterally acute. *Eye* 0.06-0.08 OCL; posterior margin of orbit entire; suborbital angle deeply curved. *Mandible* dentition formula 7-3 or 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes usually fully divided, tubercles usually small, clustered, distal margin straight.

Cephalothorax length 0.79-0.82 OCL. *Carapace* width 0.54-0.56 OCL, depth 0.56-0.61 OCL; dorsolateral bosses 0.29-0.34 OCL from eye orbit, lateral position on carapace 0.61-0.7 carapace width; cervical groove very deep, deeply rounded U in dorsal view, rarely notched, lateral setae absent.

Great chelae length 0.71-0.94 OCL, width 0.44-0.49 chelae length, depth 0.59-0.63 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.47-0.6 chelae length, depth 0.27-0.39 dactyl length, propodus length 0.35-0.46 chelae length, depth 0.52-0.75 propodus length, overlapping or crossing distally. Carpus length 1.16-1.27 carpus width, depth 0.79-0.89 carpus length, width 1.24-1.43 carpus depth; 4-7 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpus groove weak to well-developed. *Pereopod 2* length 0.83-0.92 OCL, chelae 0.34-0.36 pereopod length.

Sternal keel anterior lateral process usually shallow, distally pointed; anterior margins of processes shorter than posterior margins; processes usually meeting centrally. Median keel narrow; mesial ridge well-developed. Posterior process usually shallow, and narrow; anterior margins of processes curved, shorter than posterior margins, processes meeting centrally.

Uropod endopodite bearing single, mesial, non-terminal spine; endopod width 0.62-0.67 endopod length; telson length 0.36-0.41 OCL.

Holotype male

Antennal scale lateral margin straight; spine intermediate in strength, not produced from lateral margin; distal margin entire. *Rostrum* length 0.11 OCL, width 1.1 rostrum length; rostral profile straight in cross-section; rostral dorsal carina margin angled, apex acute; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.07 OCL; posterior margin of orbit entire; suborbital angle deeply curved. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe

long, narrow; posterolateral processes fully divided, tubercles small, clustered, distal margin straight.

Cephalothorax length 0.82 OCL. *Carapace* width 0.56 OCL, depth 0.56 OCL; dorsolateral bosses 0.34 OCL from eye orbit, lateral position on carapace 0.61 carapace width; cervical groove very deep, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.94 OCL, width 0.47 chelae length, depth 0.61 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.48 chelae length, depth 0.39 dactyl length, propodus length 0.35 chelae length, depth 0.75 propodus length, overlapping and crossing distally. Carpus length 1.16 carpus width, depth 0.86 carpus length, width 1.32 carpus depth; 4 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpus groove intermediate in depth. *Pereopod 2* length 0.89 OCL, chelae 0.36 pereopod length.

Sternal keel anterior lateral process shallow, distally pointed; anterior margins of processes shorter than posterior margins; processes meeting centrally. Median keel narrow; mesial ridge well-developed. Posterior process shallow, narrow; anterior margins of processes curved, shorter than posterior margins, processes meeting centrally.

Uropod endopodite bearing single, mesial, non-terminal spine; endopod width 0.64 endopod length; telson length 0.36 OCL.

Allotype female

Specimen as per Holotype except: *Antennal scale* spine strong, not produced from lateral margin. Dactyl and propodus overlapping distally. 5 dorsal tubercles forming distinct row on carpus. *Sternal keel* anterior lateral processes not meeting centrally.

Morphological Variation

A detailed discussion of geographical and intrapopulational variation in morphological characteristics of *O. asperrimanus* is precluded by the limited sample available; nevertheless some morphological variation is apparent.

The mandibular composition is variable, with one specimen having seven corneous denticles; the usual condition is for eight corneous denticles with third being largest. There are usually a large number of dorsal tubercles on the carpus; rarely are only four present as in the Holotype.

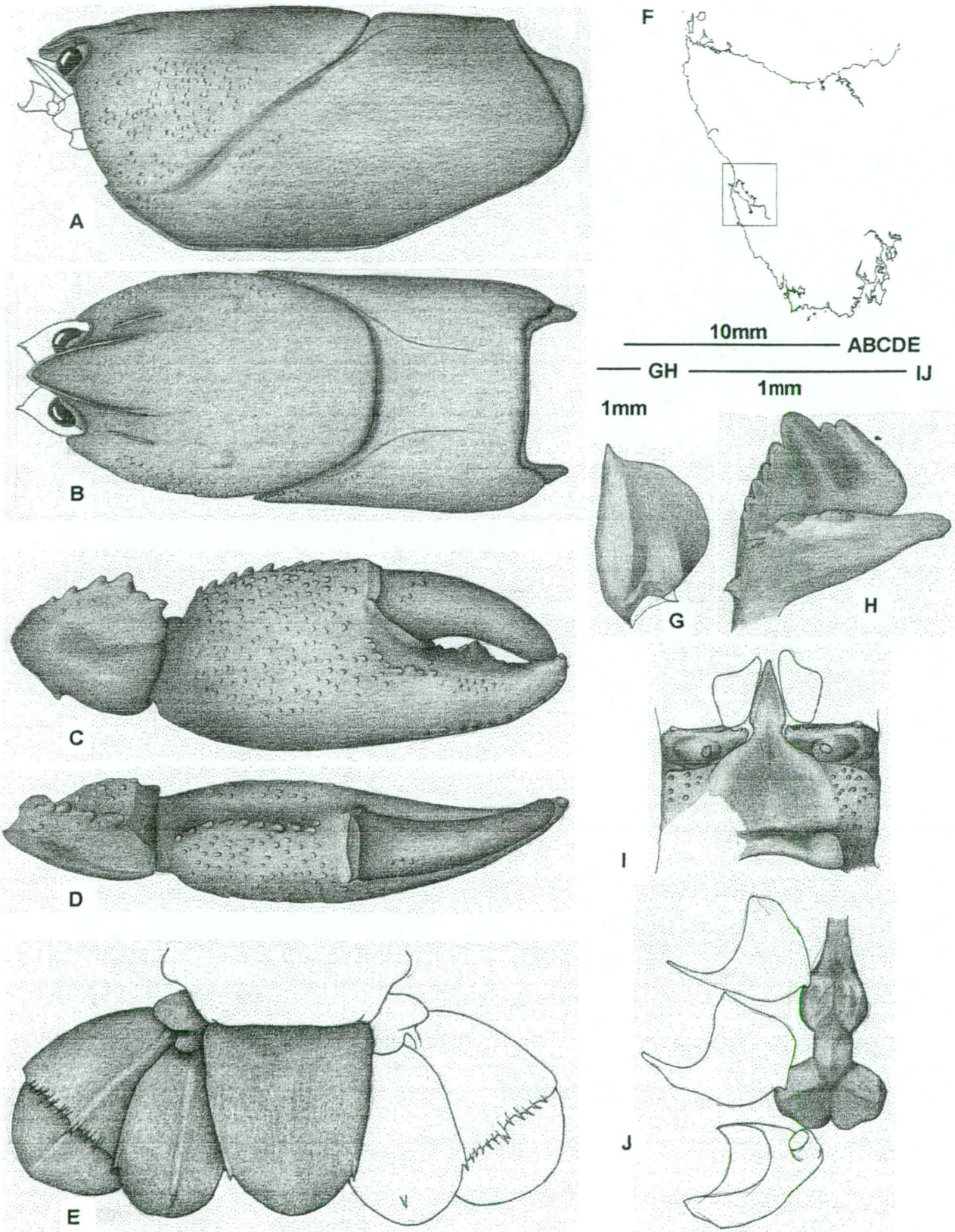


Figure 5.9. Ombrastacoides asperrimanus; *A-J from BIR9.*

***Ombrastacoides brevirostris*, sp. nov.**

(Figure 5.10)

WCT, Hansen and Richardson 1999a

Etymology: brevirostris; Latin compound adjective: "short beaked", from *brevis*, short and *rostrum*, the bill or beak, referring to the short rostrum.

Material examined

Holotype Female (ZUT BIS8) 30.26 mm OCL. The description of the habitat was the same for all specimens collected from the population, and therefore it was not possible to determine the exact habitat description for this specimen. The habitat description on the records is as follows: in deep complex peaty burrow under Melaleuca heath or from roadside gravel or from drier burrow under short heath on slope; large chimneys, often closed; at Birches Inlet, Landing Creek quarry, Tas., 7912: 736 926, 1.05.1988, AMMR, RBM, RH, PH.

Allotype Male (ZUT R6) 26.08 mm OCL, in buttongrass on muck peat on flat at Olga Valley (Line 7), Tas., 8012: 26 545, 7.02.1978, AMMR, RS, TF.

Other material examined

♂ (ZUT BIS7) 26.08 mm OCL, same data as Holotype. ♂ (ZUT BIS13) 22.66 mm OCL, same data as Holotype. ♂ (ZUT BIS14) 24.44 mm OCL, same data as Holotype. ♂ (ZUT AR5) 16.12 mm OCL, same data as Allotype. ♂ (ZUT T8) 23.66 mm OCL, same data as Allotype. ♂ (ZUT WLT13) 26.88 mm OCL, in burrow at edge of mossy rainforest creek at Indiana Creek, 500 m up road from Warners landing, Lower Gordon River, Tas., 8012: 926 863, 23.11.1984, AMMR, PHJH, RBM, RH, DS. ♂ (ZUT WLT15) 28.38 mm OCL, same data as WLT13. ♂ (ZUT WLT16) 30.00 mm OCL, same data as WLT13. ♀ (ZUT BIS5) 26.24 mm OCL, same data as Holotype. ♀ (ZUT BIS6) 26.32 mm OCL, same data as Holotype. ♀ (ZUT AR2) 17.86 mm OCL, same data as Allotype. ♀ (ZUT AR4) 26.48 mm OCL, same data as Allotype. ♀ (ZUT T7) 16.50 mm OCL, same data as Allotype. ♀ (ZUT WLT26) 19.96 mm OCL, in burrows in brown silt at edge of seepage in horizontal gully at Indiana Creek, Warners Landing, Gordon River, Tas., 8012: 926 863,

1.05.1988, AMMR, RBM, RH, PH. F (ZUT WLT29) 26.12 mm OCL, same data as WLT26. IS (ZUT WLT27) 33.12 mm OCL, same data as WLT26.

Diagnosis

Eye small, distinctively stalked; rostrum short, broad.

Description:

Antennal scale lateral margin straight; spine weak, not produced from lateral margin; distal margin usually curved. *Rostrum* length 0.4-0.94 OCL, width 1.1-3 rostrum length; rostral profile usually straight in cross-section; rostral dorsal carina usually straight, apex usually rounded; rostral lateral profile usually straight, sometimes anteriorly depressed, margin distolaterally acute. *Eye* 0.03-0.07 OCL; posterior margin of orbit usually entire; suborbital angle deeply curved to truncate. *Mandible* dentition formula 8-3 or 10-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes usually partially divided, tubercles small, clustered, distal margin usually straight.

Cephalothorax 0.81-0.85 OCL. *Carapace* width 0.47-0.64 OCL, depth 0.55-0.75 OCL; dorsolateral bosses 0.31-0.44 OCL from eye orbit, lateral position on carapace 0.75-0.91 carapace width; cervical groove deep to very deep, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 0.63-0.91 OCL, width 0.41-0.54 chelae length, depth 0.59-0.66 chelae width; chelae ventral ridge usually not extending proximal of propodus cutting surface; chelae lateral surface punctate or setose-tuberculate; chelae adductor boss weakly to strongly developed. Dactyl length 0.4-0.61 chelae length, depth. 0.29-0.42 dactyl length; propodus length 0.32-0.48 chelae length, depth 0.37-0.59 propodus length; dactyl and propodus directly opposed distally, or crossing. Carpus length 1.08-1.40 carpus width, depth 0.71-0.93 carpus length, width 1.14-1.47 carpus depth; 5 or more dorsal tubercles forming distinct row; dorsomesial tubercle row usually absent; carpus groove absent. *Pereopod 2* length 0.8-1.1 OCL, chelae 0.34-0.36 pereopod length.

Sternal keel anterior lateral process shallow, distally pointed; anterior margins of processes shorter than posterior margins; processes usually not meeting centrally. Median keel narrow to intermediate; mesial ridge well-developed. Posterior process usually deep and broad; anterior margins of processes usually curved, shorter than, or equal to posterior margins, processes meeting centrally. *Pereopod 5* lateral processes entirely visible in caudal view.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.57-0.71 endopod length; telson length 0.27-0.42 OCL.

Holotype female

Antennal scale lateral margin straight; spine weak, not produced from lateral margin; distal margin curved. *Rostrum* 0.75 OCL, width 1.5 rostrum width; rostral profile straight in cross-section; rostral dorsal carina angled, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.04 OCL; posterior margin of orbit entire; suborbital angle deeply curved. *Mandible* dentition formula 10-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin straight.

Cephalothorax length 0.82 OCL. *Carapace* width 0.48 OCL, depth 0.58 OCL; dorsolateral bosses 0.31 OCL from eye orbit, lateral position on carapace 0.84 carapace width; cervical groove very deep, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 0.75 OCL, width 0.46 chelae length, depth 0.61 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface punctate; chelae adductor boss strongly developed. Dactyl length 0.51 chelae length, depth 0.29 dactyl length; propodus length 0.42 chelae length, depth 0.45 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.32 carpus width, depth 0.76 carpus length, width 1.18 carpus depth; 6 dorsal tubercles forming distinct row; dorsomesial tubercle row

absent; carpus groove absent. *Pereopod 2* length 0.84 OCL, chelae 0.34 pereopod length.

Sternal keel anterior lateral process shallow, distally pointed; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel narrow; mesial ridge well-developed. Posterior process shallow, narrow; anterior margins of processes curved, equal in length, processes meeting centrally.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.66 endopod length; telson length 0.3 OCL.

Allotype male

As per Holotype except: *Rostrum* dorsal carina straight. *Mandible* bearing 8 corneous denticles. *Great chelae* lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl and propodus overlapping distally. Carpus with 5 dorsal tubercles forming distinct row. *Sternal keel* median keel intermediate in width. Posterior process broad; anterior margins of processes shorter than posterior margins. *Uropod* endopod mesial spine absent.

Morphological Variation

O. brevirostris is morphologically variable and much of this variation can be attributed to the wide geographic range of the species; specific regions within its range may be morphologically distinct. This is most clearly represented by the Olga Valley population (AR, R, T above) which shows the following variants. (1) The rostrum is consistently short and wide, with the dorsal carina straight, its apex rounded, profile straight in cross-section, the lateral profile anteriorly depressed and the margin distolaterally blunt. (2) The epistome tends to have a shorter, wider sagittiform, anteromedian lobe, the posterolateral processes are fully divided. (3) There is a tendency for the cervical groove to be not as deeply impressed. (4) There is a tendency for the great chelae to be longer and narrower with a more pronounced ventral ridge and weakly developed adductor boss. (5) The medial keel of the sternal

keel is narrower, the posterior processes shallower, while the anterior margins are shorter than the posterior margins. (6) The uropod endopodite rarely bears a spine. The mandible usually consists of eight corneous denticles with the third being the largest, however in the Birches Inlet population (BIS above) the mandible consists of ten corneous denticles, the third being the largest.

The blunt shape of the rostrum is distinctive, but some geographical variability is exhibited. The Birches Inlet population displays a tendency for the rostrum to be longer and more acute at the apex, however the distinctive bluntness is still apparent. At this locality the rostral lateral profile exhibits a small degree of variation in cross-section, and the dorsal carina, while usually straight, is sometimes angled.

The eye is distinctively small, and occasionally the posterior margin of the eye orbit is notched.

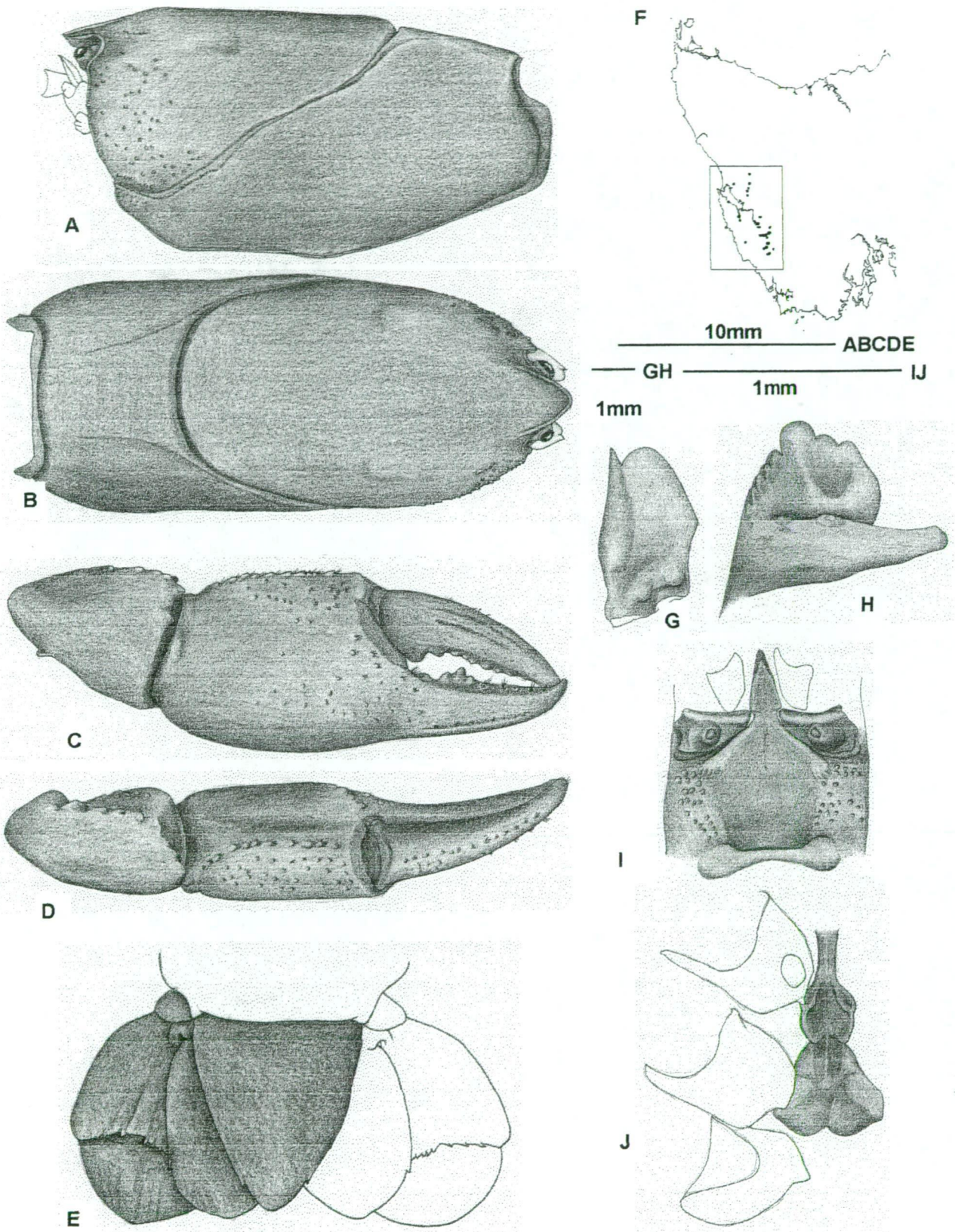


Figure 5.10. Ombrastacoides brevirostris*; A-J from BIS8.*

Ombrastacoides decemdentatus, sp. nov.

(Figure 5.11)

NT, Hansen and Richardson 1999a

Etymology: decemdentatus; Latin compound adjective: "ten-toothed", from *decem*, ten and *dentatus*, toothed; referring to the ten corneous denticles on the mandible.

Material examined

Holotype Female (ZUT NEE1) 23.34 mm OCL, in shallow burrow in roadside seepage at The Needles, Strathgordon Road, Tas., 8112: 555, 692, 22.10.1987, AMMR, PH.

Allotype Male (ZUT NEE8) 25.56 mm OCL, same data as Holotype.

Other material examined

♂ (ZUT DR11) 27.22 mm OCL, in fish trap at Lake Rhona, Denison Range, Tas., 8112: 415 886, 23.03. 1976, DC. ♂ (ZUT DR13) 21.94 mm OCL, same data as DR11. ♂ (ZUT DR36) 16.80 mm OCL, in swampy ground at east end of Lake Rhona, Denison Range, Tas, 8112: 435 835, 25.03. 1976, DC. ♂ (ZUT MSC110) 17.18 mm OCL, at Vale of Rasselas, Tas., 8112, 452 860, 20.12.1973, Project Raleigh, Denison Team. ♂ (ZUT V1122) 23.22 mm OCL, in dry tarn at Vale of Rasselas, Tas., 8112: 452 860, 17.02.1970, RS, JO. ♂ (ZUT V1163) 18.16 mm OCL, same data as V1122 except in sink hole in buttongrass. ♂ (ZUT NEE9) 24.22 mm OCL, same data as Holotype. ♂ (ZUT NEE10) 23.14 mm OCL, same data as Holotype. ♀ (ZUT DR35) 20.46 mm OCL, same data as DR36. ♀ (ZUT DR37a) 24.82 mm OCL, same data as DR36. ♀ (ZUT DR37b) 16.58 mm OCL, same data as DR36. ♀ (ZUT V1131a) 13.82 mm OCL, same data as V1122. ♀ (ZUT V1131b) 25.80 mm OCL, same data as V1122. ♀ (ZUT V1439) 24.64 mm OCL, same data as V1122. ♀ (ZUT NEE19) 23.80 mm OCL, same data as Holotype. ♀ (ZUT NEE30) 26.94 mm OCL, same data as Holotype.

Diagnosis

Mandible dentition formula 10-4; uropod endopod without mesial spine.

Description:

Antennal scale lateral margin usually straight; spine strong, not forming lateral margin; distal margin usually entire. *Rostrum* length 0.11-0.16 OCL, width 0.68-1.12 rostrum width; rostral profile concave in cross-section; rostral dorsal carina margin usually angled, apex acute; rostral lateral profile usually upturned, margin usually distolaterally acute. *Eye* 0.07-0.11; posterior margin of orbit rarely notched; suborbital angle deeply curved. *Mandible* dentition formula 10-4. *Epistome* sagittiform, anteromedian lobe usually short, wide; posterolateral processes usually fully divided, tubercles small, clustered, distal margin usually curved.

Cephalothorax length 0.79-0.86 OCL. *Carapace* width 0.5-0.59 OCL, depth 0. 57-0.65 OCL; dorsolateral bosses 0.27-0.4 OCL from eye orbit, lateral position on carapace 0.55-0.75 carapace width; cervical groove shallow to deep, usually deeply rounded U in dorsal view, lateral setae occasionally present.

Great chelae length 0.84-1.16 OCL, width 0.39-0.51 chelae length, depth 0.57-0.67 chelae width; chelae ventral ridge usually extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss usually strongly developed. Dactyl length 0.51-0.58 chelae length, depth 0.2-0.32 dactyl length; propodus length 0.37-0.48 chelae length, depth 0.39-0.69 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.04-1.48 carpus width, depth 0.68-0.96 carpus length, width 1.07-1.59 carpus depth; 3-4 dorsal tubercles forming distinct row; dorsomesial tubercle row usually present; carpal groove well-developed. *Pereopod 2* length 0.79-0.94 OCL, chelae 0.34-0.37 pereopod length.

Sternal keel anterior lateral process deep or rounded, usually distally pointed; anterior margins of processes usually shorter than posterior margins; processes not

meeting centrally. Median keel well-rounded; mesial ridge weak to well-developed. Posterior process deep, usually broad; anterior margins of processes curved, usually shorter than posterior margins; processes usually meeting centrally.

Uropod endopod mesial spine absent; endopod width 0.58-0.75 endopod length; telson length 0.36-0.42 OCL.

Holotype female

Antennal scale lateral margin straight; spine intermediate in strength, not produced from lateral margin; distal margin entire. *Rostrum* length 0.13 OCL, width 0.9 rostrum length; rostral profile concave in cross-section; rostral dorsal carina margin angled, apex acute; rostral lateral profile upturned, margin distolaterally acute. *Eye* 0.09 OCL; posterior margin of orbit entire; suborbital angle deeply curved. *Mandible* dentition formula 10-4. *Epistome* sagittiform, anteromedian lobe short, wide; posterolateral processes fully divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.8 OCL. *Carapace* width 0.54 OCL, depth 0.61 OCL; dorsolateral bosses 0.32 OCL from eye orbit, lateral position on carapace 0.59 carapace width; cervical groove shallow, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.86 OCL, width 0.43 chelae length, depth 0.59 chelae width; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.56 chelae length, depth 0.25 dactyl length; propodus length 0.46 chelae length, depth 0.49 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.26 carpus width, depth 0.79 carpus length, width 1.43 carpus depth; 4 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpal groove intermediate. *Pereopod 2* length 0.85 OCL, chelae 0.34 pereopod length.

Sternal keel anterior lateral process deep, distally pointed; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel well-rounded; mesial ridge well-developed. Posterior process deep, broad; anterior margins of processes curved, shorter than posterior margins; processes meeting centrally.

Uropod endopod mesial spine absent; endopod width 0.6 endopod length; telson length 0.36 OCL.

Allotype male

Specimen as per Holotype except: *Carpus* with 3 dorsal tubercles forming distinct row; carpal groove well-developed. *Sternal keel* anterior lateral process shallow.

Morphological Variation

Some geographic variation is evident in this species; certain characters, however, are fixed and diagnostic, for example those of the mandible, which always has ten corneous denticles, the fourth always largest.

The antennal scale of the Needles and Lake Rhona populations conform with that of the Holotype, except that the distal margin is excavate. Specimens from the Vale of Rasselas tend to have a stronger spine and a curved distal margin. Setation in the lateral portion of the cervical groove is generally absent, but usually present in specimens from Vale of Rasselas.

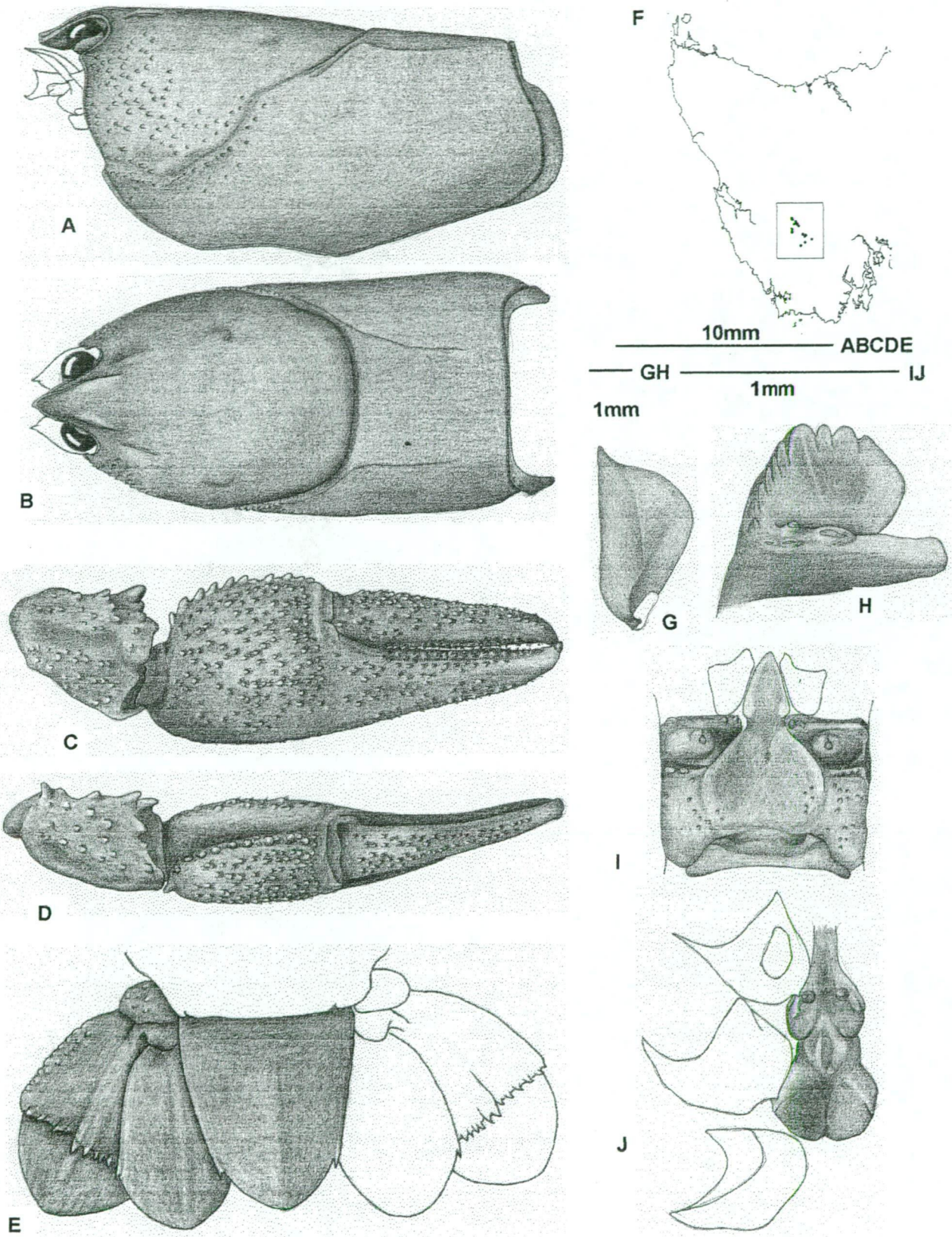


Figure 5.11. Ombrastacoides decemdentatus; *A-J from NEE1.*

***Ombrastacoides denisoni*, sp. nov.**

(Figure 5.12)

LDRT, Hansen and Richardson 1999a

Etymology: denisoni; Latin, "of the Denison"; referring to the Little Denison River near which the species is found.

Material examined

Holotype Female (ZUT LDR4) 30.58 mm OCL, in deep burrow in sandy peat under tall tea-tree sedgeland in eucalypt forest at McDougalls Road at crossing of Little Denison River, Tas., 8212: 815 425, 16.04.1988, AMMR.

Diagnosis

Uropod exopod mesial spine absent; rostrum dorsal apex rounded; carapace shallow.

Description:

Antennal scale lateral margin straight; spine strong, not produced from lateral margin; distal margin entire. *Rostrum* length 0.07 OCL, width 1.4 rostrum length; rostral profile concave in cross-section; rostral dorsal carina margin angled, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.07 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax 0.72 OCL. *Carapace* width 0.51 OCL, depth 0.51 OCL; dorsolateral bosses 0.29 OCL from eye orbit, lateral position on carapace 0.62 carapace width; cervical groove very deep, deeply rounded notched U in dorsal view, lateral setae present.

Great chelae length 0.7 OCL, width 0.51 chelae length, depth 0.7 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.55 chelae length, depth 0.32 dactyl length; propodus length 0.37 chelae length, depth 0.54 propodus length; dactyl and propodus overlapping distally. Carpus length 1.14 carpus width, depth 0.88 carpus length, width 1.16 carpus depth; 7 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpal groove weak. *Pereopod 2* length 0.82 OCL, chelae 0.35 pereopod length.

Sternal keel anterior lateral process deep, distally pointed; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel well-rounded; mesial ridge well-developed. Posterior process deep, narrow; anterior margins of processes curved, shorter than posterior margins; processes meeting centrally.

Uropod endopod mesial spine absent; endopod width 0.66 endopod length; telson length 0.36 OCL.

Morphological Variations

The restricted sample of *O. denisoni* precludes a discussion on geographic or intrapopulational variability of the morphology; however it is both genetically and morphologically distinct from other species of *Ombrastacoides*.

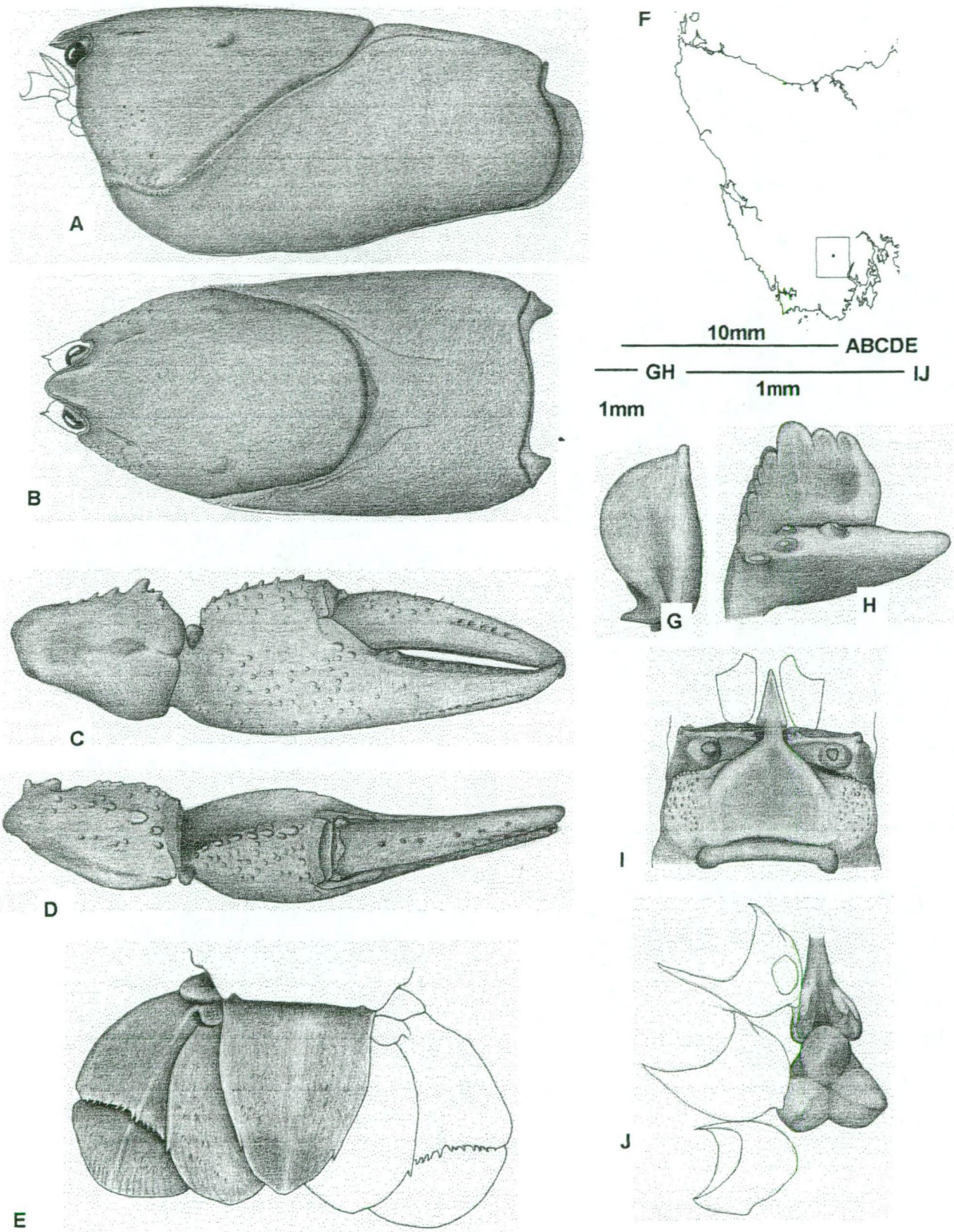


Figure 5.12. Ombrastacoides denisoni*; A-J from LDR4.*

***Ombrastacoides dissitus*, sp. nov.**

(Figure 5.13)

SET, Hansen and Richardson 1999a

Etymology: dissitus; Latin adjective; "remote", referring to the distribution of the species in far southeast Tasmania.

Material examined

Holotype Female (ZUT SET9) 22.20 mm OCL, in deep burrow in clay in burnt eucalypt forest, now Restio, Stipa, Ghania sedgeland at plain east of junction of Leprena track with Catamaran River, Lune River, Tas., 8211: 920 875, 26.03.1988, AMMR.

Allotype Male (ZUT SET11) 17.64 mm OCL, same data as Holotype.

Paratypes. ♂ (ZUT V1119) 22.78 mm OCL, halfway across Cockle Creek Plain, South Coast Track, 8210: 895 731, 1.02.1970, ISW, JO. ♂ (ZUT V1120) 18.00 mm OCL, same data as V1119. ♀ (ZUT SET6) 21.64 mm OCL, same data as Holotype. ♀ (ZUT SET8) 26.48 mm OCL, same data as Holotype.

Diagnosis

Rostrum dorsal carina apex rounded; great chelae ventral margin ridge not extending proximal of propodus cutting surface; sternal keel ridge not well developed.

Description:

Antennal scale lateral margin straight; spine weak to strong, not forming lateral margin; distal margin curved. *Rostrum* length 0.07-0.13 OCL, width 0.85-1.34 rostrum width; rostral profile concave in cross-section; rostral dorsal carina straight, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.06-0.1 OCL; posterior margin of orbit usually entire; suborbital angle deeply curved. *Mandible* dentition formula 8-3, 10-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.7-0.84 OCL. *Carapace* width 0.52-0.54 OCL, depth 0.61-0.67 OCL; dorsolateral bosses 0.29-0.37 OCL from eye orbit, lateral position on carapace 0.7-0.83 carapace width; cervical groove shallow to deep, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 0.74-0.89 OCL, width 0.41-0.48 chelae length, depth 0.66-0.75 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.54-0.57 chelae length, depth 0.3-0.35 dactyl length; propodus length 0.37-0.44 chelae length, depth 0.47-0.57 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.08-1.24 carpus width, length 0.81-0.93 carpus length, width 1.16-1.38 carpus depth; 5-7 dorsal tubercles greater forming distinct row; dorsomesial tubercle row usually present; carpal groove weak to well-developed. *Pereopod 2* length 0.83-0.92 OCL, chelae 0.34-0.36 pereopod length.

Sternal keel anterior lateral process shallow to deep, distally pointed; anterior margins of processes shorter than posterior margins; processes usually not meeting centrally. Median keel well-rounded; mesial ridge not well-developed. Posterior process deep, broad; anterior margins of processes curved, shorter than posterior margins; processes meeting centrally.

Uropod endopod mesial spine absent; endopod width 0.6-0.67 endopod length; telson length 0.33-0.39 OCL.

Holotype female

Antennal scale lateral margin straight; spine intermediate in strength, not produced from lateral margin; distal margin curved. *Rostrum* 0.13 OCL, width 0.85 rostrum length; rostral profile concave in cross-section; rostral dorsal carina straight, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.07; posterior margin of orbit entire; suborbital angle deeply curved. *Mandible*

dentition formula 10-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.68 OCL. *Carapace* width 0.53 OCL, depth 0.65 OCL; dorsolateral bosses 0. 34 OCL from eye orbit, lateral position on carapace 0.7 carapace width; cervical groove deep, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 0.86 OCL, width 0.48 chelae length, depth 0.66; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.54 chelae length, depth 0.34 dactyl length; propodus length 0.37 chelae length, depth 0.57 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.08 carpus width, depth 0.93 carpus length, width 1.31 carpus depth; 7 dorsal tubercles greater forming distinct row; dorsomesial tubercle row present; carpal groove well-developed. *Pereopod 2* length 0.81 OCL, chelae 0.35 pereopod length.

Sternal keel anterior lateral process deep, distally pointed; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel well-rounded; mesial ridge not well-developed. Posterior process deep, broad; anterior margins of processes curved, shorter than posterior margins; processes meeting centrally. *Pereopod 5* lateral processes entirely visible in caudal view.

Uropod endopod mesial spine absent; endopod width 0.64 endopod length; telson length 0.39 OCL.

Allotype male

Specimen as per Holotype except: *Antennal scale* spine weak. *Mandible* dentition formula 8-3. *Carapace* cervical groove shallow. *Carpus* with 5 dorsal tubercles

forming distinct row. *Sternal keel* anterior lateral process shallow. *Uropod* endopodite bearing single, non-terminal mesial spine.

Morphological Variation

Due to the restricted geographical range of *O. dissitus* (and the small number of specimens available), only intrapopulation variation can be discussed.

The mandible varies markedly; half of the specimens had less than eight corneous denticles, whereas the others had eight (in all specimens the third denticle was the largest). The tailfan is somewhat variable in shape and spination; typically the uropod exopodite does not bear a spine, but one is occasionally present.

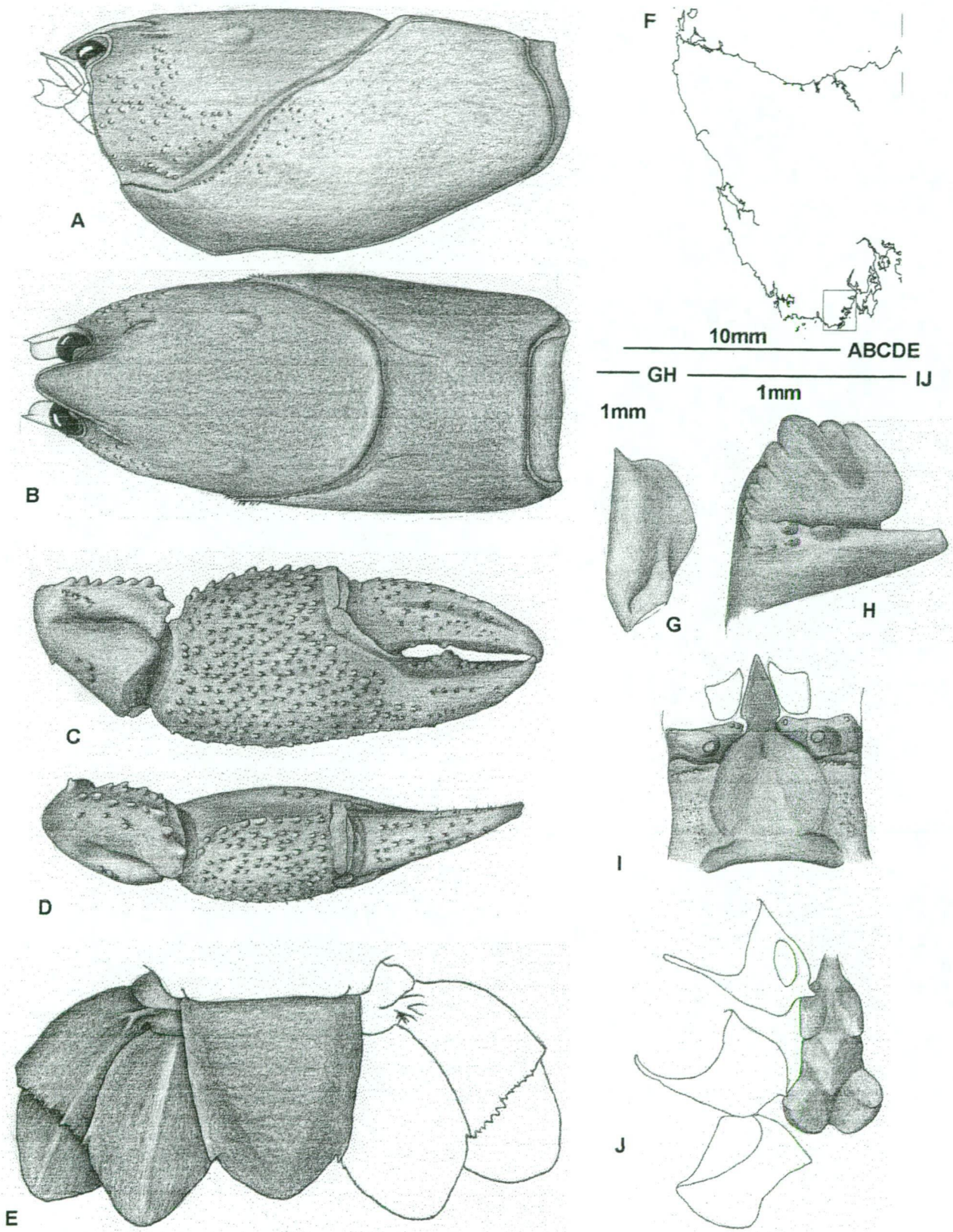


Figure 5.13. Ombrastacoides dissitus*; A-J from SET9.*

***Ombrastacoides huonensis*, sp. nov.**

(Figure 5.14)

SPTA, Hansen and Richardson 1999a

Etymology: huonensis; Latin, "of the Huon", referring to the Huon River, near which the species is found.

Material examined

Holotype Male (ZUT HHT10) 21.72 mm OCL, in buttongrass heath on slope at plain west of Scotts Peak Road near Harlequin Hill, Tas., 8112: 475 425, 4.03.1983, AMMR, RS.

Allotype Female (ZUT CSRT19) 25.70 mm OCL, in heath and buttongrass on flat at Crossing River at Port Davey Track crossing, Tas., 8111: 324 271, 1.11.1981, AMMR, RS, DAR, RH.

Other material examined

♂ (ZUT CRST15) 31.56 mm OCL, same data as Allotype. ♂ (ZUT CRST16) 25.28 mm OCL, same data as Allotype. ♂ (ZUT HHT12) 27.72 mm OCL, same data as Holotype. ♂ (ZUT HHT15) 26.10 mm OCL, same data as Holotype. ♀ (ZUT CRST14) 26.18 mm OCL, same data as Allotype. ♀ (ZUT CRST 18) 26.26 mm OCL, same data as Allotype. ♀ (ZUT HHT2) 22.90 mm OCL, same data as Holotype. ♀ (ZUT HHT7) 26.86 mm OCL, same data as Holotype. ♀ (ZUT HHT11) 21.58 mm OCL, same data as Holotype. IS (ZUT CRST7) 18.88 mm OCL, same data as Allotype.

Diagnosis

Rostrum long, dorsal carina angled; antennal scale distal margin curved; chelae dactyl and propodus crossing.

Description:

Antennal scale lateral margin straight; spine weak to strong, not produced from lateral margin; distal margin curved. *Rostrum* length 0.11-0.14 OCL, width 0.67-0.84 rostrum length; rostral profile concave in cross-section; rostral dorsal carina angled, apex acute; rostral lateral profile usually anteriorly depressed, margin distolaterally acute. *Eye* 0.07-0.09 OCL; posterior margin of orbit usually notched; suborbital angle deeply curved. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe usually short, wide; posterolateral processes usually partially divide, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.8-0.85 OCL. *Carapace* width 0.52-0.56 OCL, depth 0.61-0.65 OCL; dorsolateral bosses 0.31-0.35 OCL from eye orbit, lateral position on carapace 0.56-0.7 carapace width; cervical groove usually shallow, deeply rounded U in dorsal view, rarely notched, lateral setae absent.

Great chelae length 0.87-1.02 OCL, width 0.44-0.53 chelae length, depth 0.56-0.61 chelae width; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface tuberculate to setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.52-0.6 chelae length, depth 0.24-0.32 dactyl length; propodus length 0.21-0.5 chelae length, depth 0.46-1.2 propodus length; dactyl and propodus overlapping distally and crossing. Carpus length 1.05-1.16 carpus width, depth 0.83-0.95 carpus length, width 1.24-1.51 carpus depth; 3-5 dorsal tubercles usually forming distinct row; dorsomesial tubercle row usually present; carpal groove well-developed. *Pereopod 2* length 0.74-0.92 OCL, chelae 0.34-0.38 pereopod length.

Sternal keel anterior lateral process shallow, usually distally rounded; anterior margins of processes usually shorter than posterior margins; processes usually not meeting centrally. Median keel narrow to intermediate in width; mesial ridge well-developed. Posterior process deep, broad; anterior margins of processes curved, shorter than posterior margins; processes meeting centrally.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.61-0.78 endopod length; telson length 0.38-0.43 OCL.

Holotype male

Antennal scale lateral margin straight; spine intermediate in strength, not produced from lateral margin; distal margin curved. *Rostrum* length 0.13 OCL, width 0.78 rostrum length; rostral profile concave in cross-section; rostral dorsal carina angled, apex acute; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.09 OCL; posterior margin of orbit notched; suborbital angle deeply curved. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe short, wide; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.82 OCL. *Carapace* width 0.54 OCL, depth 0.65 OCL; dorsolateral bosses 0.34 OCL from eye orbit, lateral position on carapace 0.56 carapace width; cervical groove shallow, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.94 OCL, width 0.52 chelae length, depth 0.6 chelae width; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.52 chelae length, depth 0.32 dactyl length; propodus length 0.41 chelae length, depth 0. 6 propodus length; dactyl and propodus overlapping distally and crossing. Carpus length 1.12 carpus width, depth 0.89 carpus length, width 1.34 carpus depth; 5 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpal groove well-developed. *Pereopod 2* length 0.83 OCL, chelae 0.37 pereopod length.

Sternal keel anterior lateral process shallow, distally rounded; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel intermediate in width; mesial ridge well-developed. Posterior process deep,

broad; anterior margins of processes curved, shorter than posterior margins; processes meeting centrally.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.69 endopod length; telson length 0.41 OCL.

Allotype female

Specimen as per Holotype except: *Epistome* sagittiform, anteromedian lobe long, narrow. *Great chelae* lateral surface tuberculate. *Sternal keel* median keel narrow.

Morphological Variation

P. huonensis exhibits little morphological variability. Minor differences, as suggested by the variation between the Holotype and Allotype, occur to some extent randomly over the species' range, and no pattern is discernable.

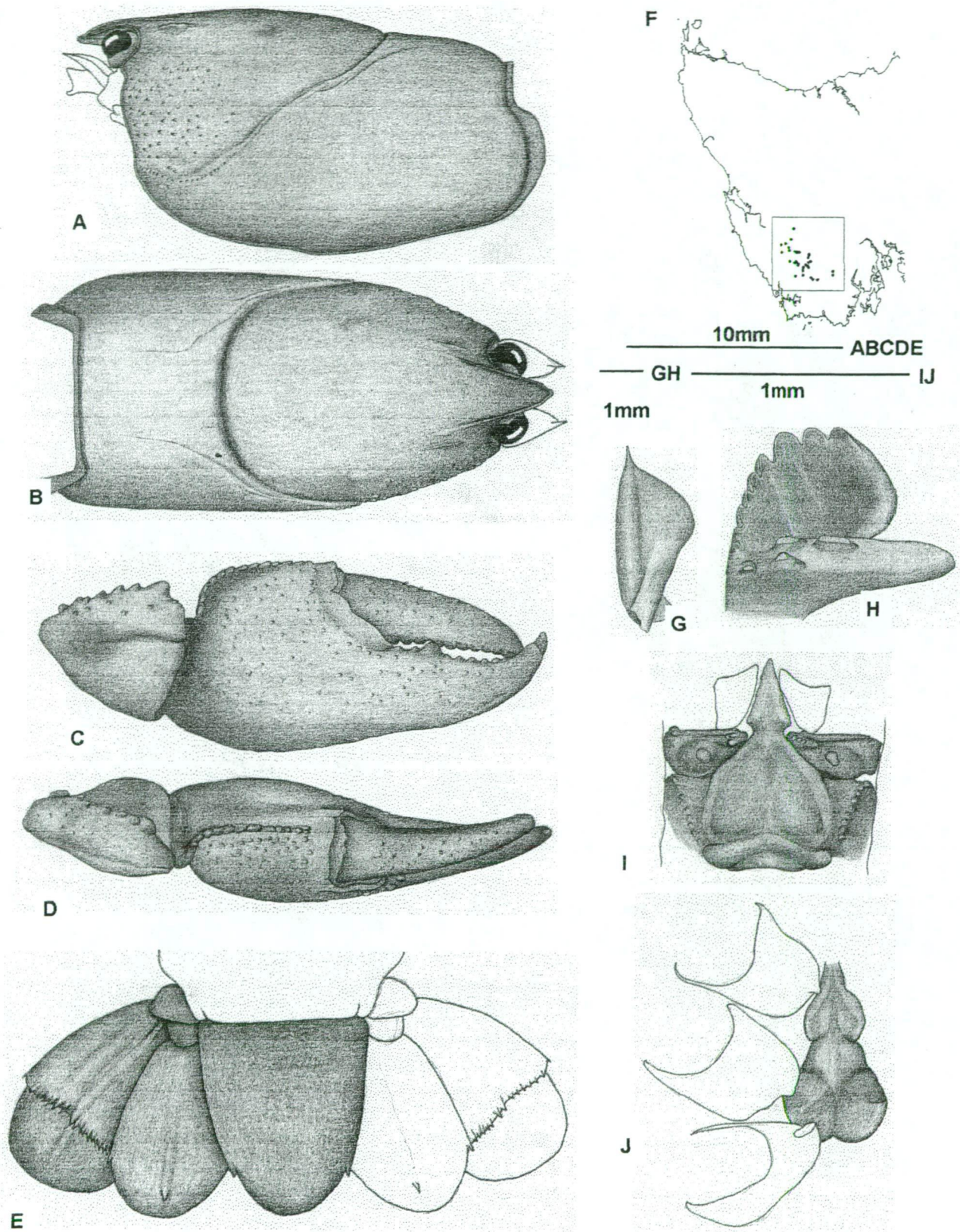


Figure 5.14. Ombrastacoides huonensis*; A-J from HHT10.*

***Ombrastacoides ingressus*, sp. nov.**

(Figure 5.15)

VPT, Hansen and Richardson 1999a

Etymology: ingressus; Latin, "an entrance", referring to the distribution of the species in the vicinity of Victoria Pass.

Holotype Male (ZUT VP8) 22.12 mm OCL, in wide complex burrow in floodplain of small creek at east side of Victoria Pass, Lyell Highway, Tas., 8013: 992 367, 26.11.1984, AMMR, PHJH, DS.
Allotype Female (ZUT VP13) 23.02 mm OCL, same data as VP8.

Other material examined

♂ (ZUT VP11) 25.68 mm OCL, same data as VP8. ♂ (ZUT VP21) 27.66 mm OCL, same data as VP8. ♀ (ZUT VP15) 26.72 mm OCL, same data as VP8. ♀ (ZUT VP16) 27.70 mm OCL, same data as VP8.

Diagnosis:

Rostrum profile straight in cross-section; great chelae ventral margin ridge not extending proximal of propodus cutting surface; antennal scale spine strong; epistome posterolateral processes partially separated, distal margins curved; carapace wide.

Description:

Antennal scale lateral margin straight; spine strong, usually forming lateral margin, occasionally not; distal margin excavate. *Rostrum* length 0.09-0.11 OCL, width 0.9-1.4 rostrum length; rostral profile straight in cross-section; rostral dorsal carina margin angled, apex acute; rostral lateral profile straight or anteriorly depressed, margin distolaterally acute. *Eye* 0.05-0.08 OCL; posterior margin of orbit usually entire; suborbital angle deeply curved to truncate. *Mandible* dentition formula 8-3, rarely 10-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles usually large, discrete, distal margin curved.

Cephalothorax length 0.76-0.81 OCL. *Carapace* width 0.51-0.55 OCL, depth 0.57-0.6 carapace width; dorsolateral bosses 0.28-0.53 OCL from eye orbit, lateral position 0.62-0.69 carapace width; cervical groove deep to very deep, deeply rounded U in dorsal view, often notched, lateral setae usually absent.

Great chelae length 0.8-1.11 OCL, width 0.27-0.5 chelae length, depth 0.61-0.65 chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.35-0.58 chelae length, depth 0.3-0.38 dactyl length; propodus length 0.28-0.44 chelae length, depth 0.45-0.57 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.16-1.36 carpus width, depth 0.72-0.86 carpus length, width 1.25-1.37 carpus depdth; 3-5 dorsal tubercles forming distinct row; dorsomesial tubercle row usually present; carpal groove weak to well-developed. *Pereopod 2* length 0.84-0.89 OCL, chelae 0.35-0.37 pereopod length.

Sternal keel anterior lateral process deep, distally rounded; anterior and posterior margins of processes equal; processes usually not meeting centrally. Median keel intermediate to well-rounded; mesial ridge usually well-developed, occasionally not. Posterior process shallow, narrow to broad; anterior margins of processes curved, anterior and posterior margins equal, processes usually meeting centrally.

Uropod endopodite sometimes bearing single, non-terminal spine; endopod width 0.66-0.78 endopod length; telson length 0.34-0.4 OCL.

Allotype female

Antennal scale lateral margin straight; spine intermediate in strength, not produced from lateral margin; distal margin excavate. *Rostrum* length 0.07 OCL, width 1.36 rostrum length; rostral profile straight in cross-section; rostral dorsal carina margin angled, apex acute; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.06 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe long,

narrow; posterolateral processes partially divided, tubercles large, discrete, distal margin curved.

Cephalothorax length 0.63 OCL. *Carapace* width 0.52 carapace length, depth 0.56 carapace OCL; dorsolateral bosses 0.31 OCL from eye orbit, lateral position 0.62 carapace width; cervical groove deep, deeply rounded notched U in dorsal view, lateral setae absent.

Great chelae length 0.78 OCL, width 0.42 chelae length, depth 0.7chelae width; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.6 chelae length, depth 0.28 dactyl length; propodus length 0.43 chelae length, depth 0.48 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.24 carpus width, depth 0.81 carpus length, width 1.37 carpus depth; 3 dorsal tubercles forming distinct row; dorsomesial tubercle row absent; carpal groove weak. *Pereopod 2* length 0.9 OCL, chelae 0.36 pereopod length.

Sternal keel anterior lateral process deep, distally rounded; anterior and posterior margins of processes equal; processes not meeting centrally. Median keel well-rounded; mesial ridge not well-developed. Posterior process shallow, broad; anterior margins of processes curved, anterior and posterior margins equal, processes meeting centrally.

Uropod endopodite bearing single, non-terminal spine; endopod width 0.73 endopod length; telson length 0.34 OCL.

Morphological Variation

Due to the restricted range of the samples geographical variation cannot be readily discussed, nevertheless several intrapopulational variations in morphological characters are evident.

Whilst rostral shape varies somewhat from the Allotype, it is remarkably consistent in the other specimens. Only one specimen exhibited any variation in the composition of the epistome; on this specimen the posterolateral process tubercles were small and clustered. Though not always present, it is not uncommon to find a dorsomesial row of tubercles on the great chelae carpus.

The tailfan is quite variable in shape; the uropod endopodite occasionally lacks a mesial spine.

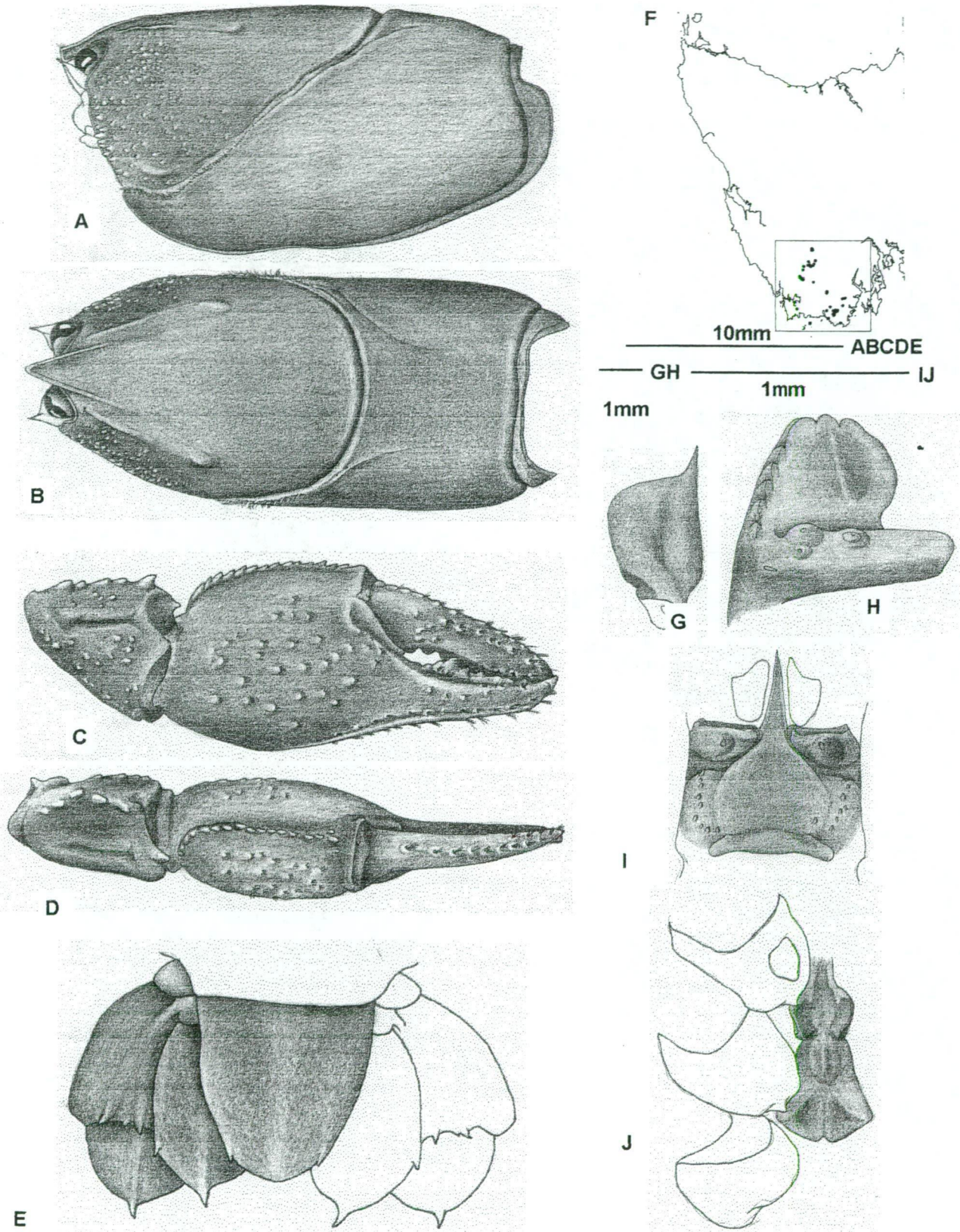


Figure 5.15. Ombrastacoides ingressus; *A-J from VP21.*

***Ombrastacoides parvicaudatus*, sp. nov.**

(Figure 5.16)

LMT, Hansen and Richardson 1999a

Etymology: parvicaudatus; Latin compound adjective; "short-tailed", from *parvus*, small and *cauda*, the tail, referring to the relatively short telson of this species.

Material examined

Holotype Female (ZUT V1471) 12.38 mm OCL, at creek near King River, Lyell Highway, Tas., 8013: 885 417, 17.01.1970, ISW.

Allotype Male (ZUT MSC133a) 14.32 mm OCL, in shallow burrow under rocks at edge of creek at tributary of King River above Lyell Highway, Tas., 8013: 883 424, 3.05.1988, PH.

Paratypes ♂ (ZUT MSC 133b) 13.12 mm OCL, same data as Allotype. ♂ (ZUT V1470) 12.04 mm OCL, same data as Holotype. ♀ (ZUT MSC134) 18.86 mm OCL, same data as Allotype. ♀ (ZUT V1256) 24.76 mm OCL, at Comstock Creek, King River valley, Tas., 8013: 880 445, 4.11.1975, PS et al.

Diagnosis

Carapace narrow; cervical groove lateral setae absent; rostrum cross-section profile straight; carpal groove present.

Description:

Antennal scale lateral margin straight; spine strong, not produced from lateral margin; distal margin entire. *Rostrum* length 0.1-0.13 OCL, width 0.8-1.4 rostrum length; rostral profile straight in cross-section; rostral dorsal carina angled, apex acute; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.07-0.1 OCL; posterior margin of orbit rarely notched; suborbital angle deeply curved. *Mandible* dentition formula 8-3, 10-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes usually partially divided, tubercles small, clustered, distal margin usually curved.

Cephalothorax length 0.77-0.84 OCL. *Carapace* width 0.46-0.49 OCL, depth 0.56-0.59 OCL; dorsolateral bosses 0.28-0.36 OCL from eye orbit, lateral position on carapace 0.65-0.73 carapace width; cervical groove deep, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.81-0.94 OCL, width 0.41-0.46 chelae length, depth 0.66-0.7; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss usually weakly developed. Dactyl length 0.49-0.54 chelae length, depth 0.31-0.38 dactyl length; propodus length 0.34-0.43 chelae length, depth 0.46-0.68 propodus length; dactyl and propodus overlapping distally. Carpus length 1.12-1.29 carpus width, depth 0.78-0.89 carpus length, width 1.26-1.37 carpus depth; 5-6 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpal groove weak to well-developed. *Pereopod 2* length 0.85-0.95 OCL, chelae 0.34-0.38 pereopod length.

Sternal keel anterior lateral process deep, distally usually rounded; anterior margins of processes usually shorter than posterior margins; processes usually meeting centrally. Median keel intermediate in width; mesial ridge well-developed. Posterior process deep, narrow; anterior margins of processes straight or curved, usually shorter than posterior margins; processes meeting or not meeting centrally.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.56-0.70 endopod length; telson length 0.34-0.37 OCL.

Holotype female

Antennal scale lateral margin straight; spine strong, not produced from lateral margin; distal margin entire. *Rostrum* length 0.13 OCL, width 0.8 rostrum length; rostral profile straight in cross-section; rostral dorsal carina angled, apex acute; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.09 OCL; posterior margin of orbit entire; suborbital angle deeply curved. *Mandible* dentition

formula 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.82 OCL. *Carapace* width 0.49 OCL, depth 0.59 OCL; dorsolateral bosses 0.36 OCL from eye orbit, lateral position on carapace 0.7 carapace width; cervical groove deep, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.85 OCL, width 0.45 chelae length, depth 0.7; chelae ventral ridge not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.54 chelae length, depth 0.32 dactyl length; propodus length 0.37 chelae length, depth 0.53 propodus length; dactyl and propodus overlapping distally. Carpus 1.27 carpus width, depth 0.78 carpus length, width 1.37 carpus depth; 5 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpal groove intermediate in depth. *Pereopod 2* length 0.87 OCL, chelae 0.36 pereopod length.

Sternal keel anterior lateral process deep, distally rounded; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel intermediate in width; mesial ridge well-developed. Posterior process deep, narrow; anterior margins of processes curved, shorter than posterior margins; processes not meeting centrally.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.7 endopod length; telson length 0.37 OCL.

Allotype male

Specimen as per Holotype except: *Great chelae* 6 dorsal tubercles forming distinct row; carpal groove weak. *Sternal keel* anterior lateral processes meeting centrally.

Morphological Variation

Little information on a species' morphological variability can be ascertained from a sample of six specimens; nevertheless several morphological characters varied between the specimens. The mandibular structure conforms with the Allotype in four specimens, but in two ten corneous denticles are present.

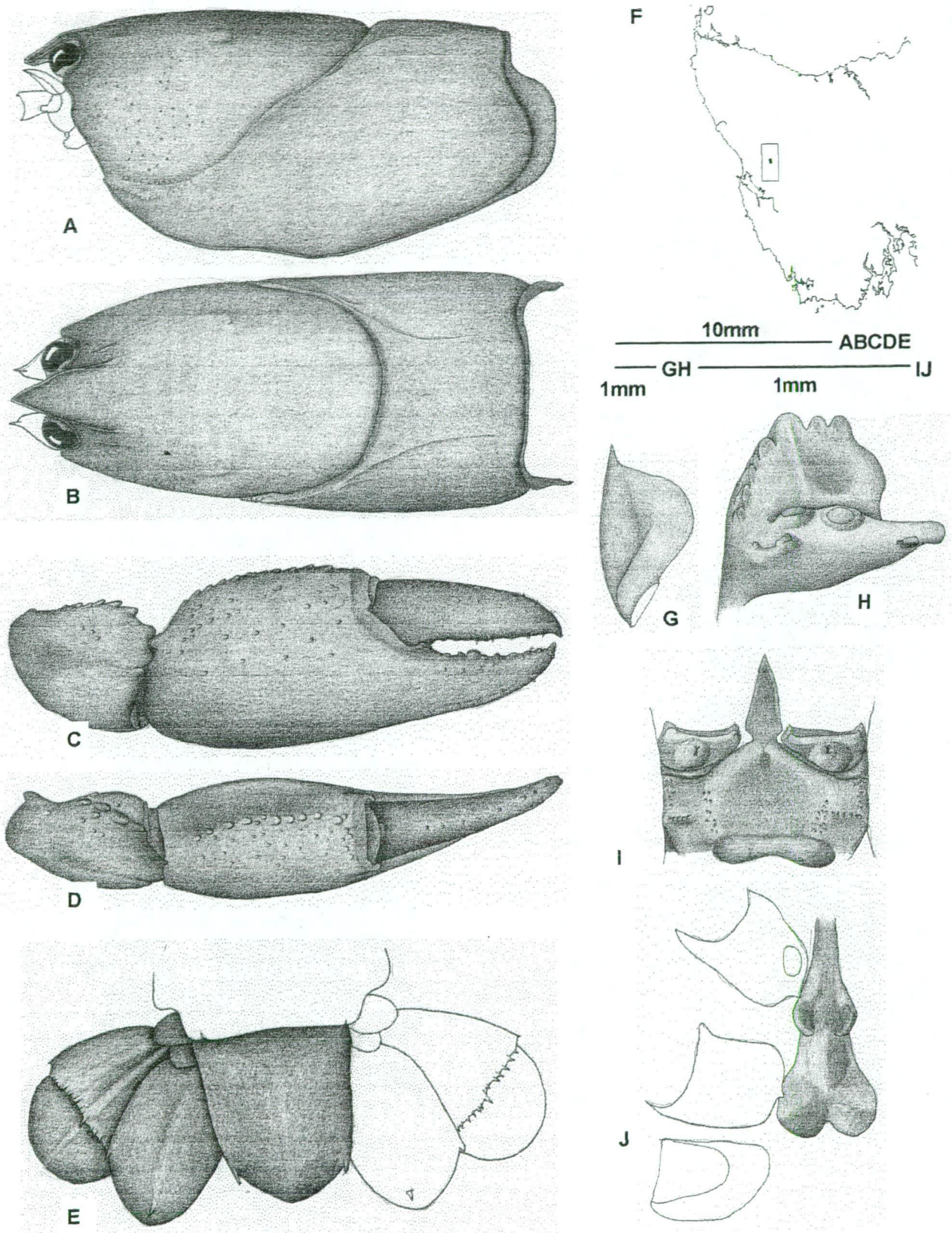


Figure 5.16. Ombrastacoides parvicaudatus*; A-J from V1471.*

***Ombrastacoides professorum*, sp. nov.**

(Figure 5.17)

ACT, Hansen and Richardson 1999a

Etymology: professorum; Latin, "of the professors", referring to the Professors Range in which the species is found. Peaks are named after Darwin, Lyell, Sedgwick, Owen, Huxley and Jukes.

Material examined

Holotype Male (ZUT V1311) 25.62 mm OCL, in buttongrass at Allens Creek, Crotty Road, behind helipad, Tas., 8013: 855 228, 16.03.1974, CR, AB-M, WW, MB.

Allotype Female (ZUT V1276) 24.18 mm OCL, same data as Holotype.

Paratypes. ♂ (ZUT V1322) 17.82 mm OCL, same data as Holotype; ♂ (ZUT V1313) 19.00 mm OCL, same data as Holotype; ♀ (ZUT V1304) 15.26 mm OCL, same data as Holotype; ♀ (ZUT V1314) 28.72 mm OCL, same data as Holotype.

Diagnosis

Antennal scale spine forming lateral margin; epistome posterolateral processes fully divided.

Description:

Antennal scale lateral margin usually curved; spine strong, produced from lateral margin; distal margin entire. *Rostrum* length 0.09-0.14OCL, width 0.88-1.14 rostrum length; rostral profile straight in cross-section; rostral dorsal carina usually angled, apex acute; rostral lateral profile usually anteriorly depressed, margin distolaterally acute. *Eye* 0.05-0.1 OCL; posterior margin of orbit entire; suborbital angle deeply curved to truncate. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes fully divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.79-0.85 OCL. *Carapace* width 0.5-0.53 OCL, depth 0.58-0.61 OCL; dorsolateral bosses 0.3-0.37 from eye orbit, lateral position on carapace

0.71-0.76 carapace width; cervical groove very deep, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.75-0.82 OCL, width 0.45-0.54 chelae length, depth 0.6-0.66; chelae ventral ridge sometimes not extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.52-0.55 chelae length, depth 0.29-0.38 dactyl length; propodus length 0.36-0.44 chelae length, depth 0.51-0.71 propodus length; dactyl and propodus usually directly opposed distally, tips not overlapping. Carpus length 1.11-1.32 carpus width, depth 0.76-0.9 carpus length, width 1.3-1.37 carpus depth; 4-5 dorsal tubercles forming distinct row; dorsomesial tubercle row usually present; carpal groove absent. *Pereopod 2* length 0.79-0.86 OCL, chelae 0.33-0.36 pereopod length.

Sternal keel anterior lateral process shallow, distally pointed; anterior margins of processes shorter than posterior margins; processes often not meeting centrally. Median keel intermediate to well-rounded; mesial ridge well-developed. Posterior process shallow to deep, broad; anterior margins of processes straight or curved, shorter than posterior margins; processes meeting centrally.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.64-0.72 endopod length; telson length 0.36-0.38 OCL.

Holotype Male

Antennal scale lateral margin curved; spine strong, produced from lateral margin; distal margin entire. *Rostrum* length 0.11 OCL, width 0.9 rostrum length; rostral profile straight in cross-section; rostral dorsal carina angled, apex acute; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.06 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes fully divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.82 OCL. *Carapace* width 0.52 OCL, depth 0.58 OCL; dorsolateral bosses 0.34 OCL from eye orbit, lateral position on carapace 0.73

carapace width; cervical groove very deep, deeply rounded U in dorsal view, lateral setae absent.

Great chelae length 0.8 OCL, width 0.45 chelae length, depth 0.6; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.55 chelae length, depth 0.29 dactyl length; propodus length 0.44 chelae length, depth 0.51 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.32 carpus width, depth 0.76 carpus length, width 1.41 carpus depth; 5 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpal groove absent. *Pereopod 2* length 0.86 OCL, chelae 0.36 pereopod length.

Sternal keel anterior lateral process shallow, distally pointed; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel intermediate in width; mesial ridge well-developed. Posterior process deep, broad; anterior margins of processes curved, shorter than posterior margins; processes meeting centrally.

Uropod endopodite bearing single, non-terminal mesial spine; endopod width 0.64 endopod length; telson length 0.37 OCL.

Allotype Female

As per Holotype except: *Rostrum* dorsal carina straight. *Great chelae* ventral ridge not extending proximal of propodus cutting surface. Dactyl and propodus overlapping distally. Carpus groove weak.

Morphological Variation

Due to the restricted range and sample size of this species, geographical variation cannot be discussed, whilst little of the morphological variation can be gauged. The antennal scale was consistent across the specimens with one exception, in which the lateral scale was straight rather than curved. Minor variations occur in the form of the rostrum, but the usual form is that displayed by the Holotype.

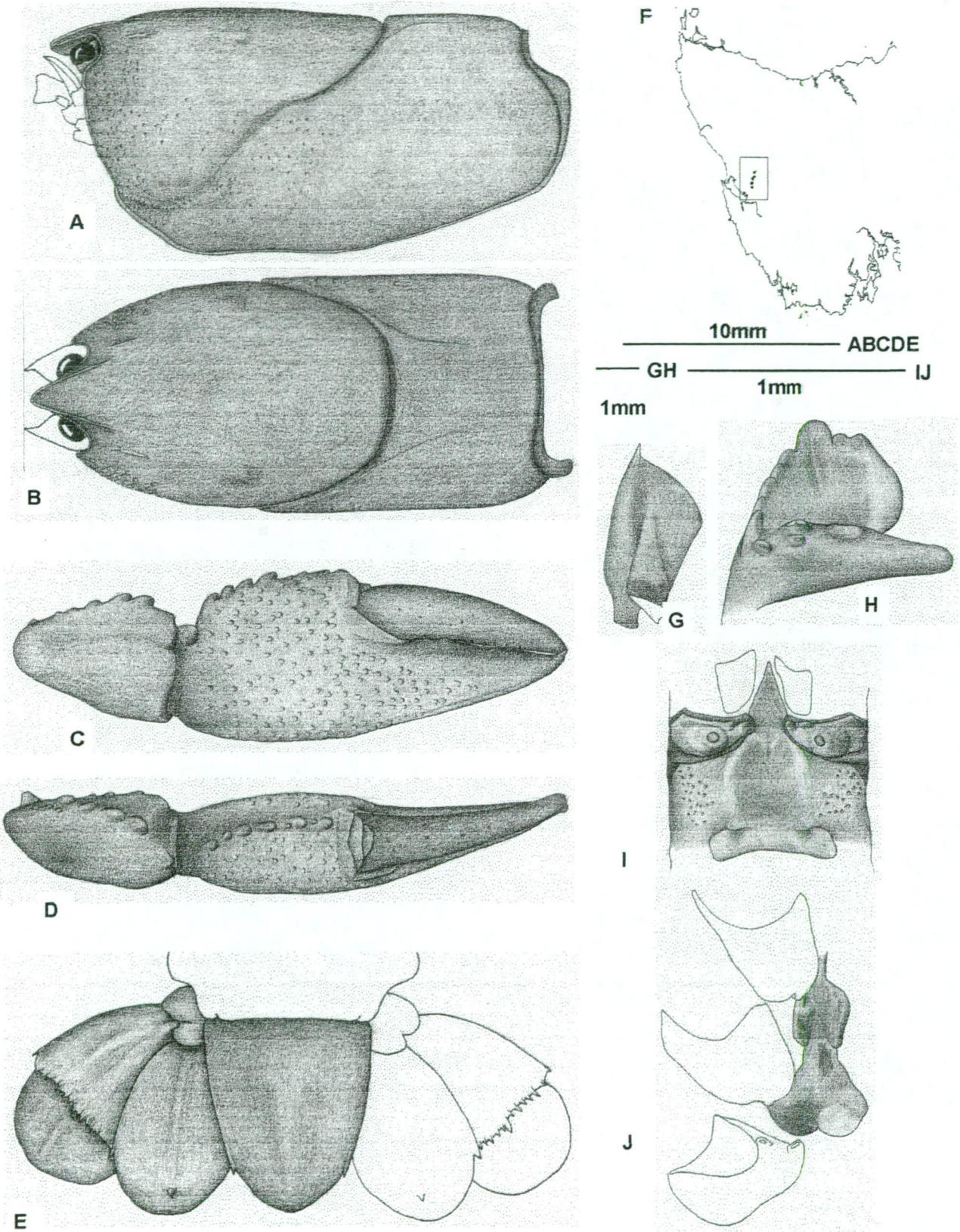


Figure 5.17. Ombrastacoides professorum*; A-J from V1311.*

Order DECAPODA
Infraorder ASTACIDEA
Superfamily PARASTACIDEA
Family PARASTACIDAE
Genus *Spinastacoides*, gen nov.

Etymology. Spinastacoides; compound noun; "spiny crayfish", from *spina*, (Greek) a spine and *Astacoides*, (Latin) crayfish-like, referring to the uropod exopod terminal spine. Gender: masculine.

Type species: *Syntype Male* (NMV J899) 22 mm OCL, in buttongrass at New Harbour, Tas., 27.01.1926 (C. King).

Description

Total length rarely more than 80mm. Antennae at least length of carapace, inner flagellum of antennule shorter than outer flagellum. *Antennal scale* lateral margin straight or curved; spine weak to strong, not producing from lateral margin; distal margin entire, excavate, or curved. *Rostrum* short and wide to long and narrow; rostral cross-section profile straight, concave; rostral dorsal carina margin straight or angled, apex rounded to acute; rostral lateral profile anteriorly depressed, straight, or upturned, rostrum distally blunt to acute. *Eye* usually large; eye orbit posterior margin notched or entire; suborbital angle deeply curved to truncate. *Mandible* bearing 7-10 corneous denticles, numbers 3 or 4 largest, dentition formula usually 8-3 or 10-4. *Epistome* sagittiform, anteromedian lobe short and wide to long and narrow; posterolateral processes either partially or fully divided, tubercles on processes ranging from large and discrete to small and clustered, distal margins curved to straight.

Cephalothorax and *Carapace* variable in length, width and depth, setose, tuberculate or both, anteroventral cephalon more coarsely ornamented than branchiostegites; position of dorsolateral bosses variable both in distance from eye orbit, and in relation to carapace width; cervical groove impression shallow to very deep, dorsally with deeply rounded U-shape (sometimes notched); cervical groove lateral setae present. Anterolateral extension of branchiocardiac grooves distinct but close to

cervical groove. Areola broad. Cervical and branchiocardiac grooves close, but obviously separated.

Great chelae variable in length (but approximate with OCL), width and depth; with distinctive ventral ridge; chelae lateral propodal surface setose-tuberculate; propodus adductor boss development weak to strong. Dactyl and propodus variable in length and depth; dactyl and propodus meeting or crossing, overlappping. Carpus variable in length, width and depth; 3 to 7 dorsal tubercles usually forming distinct row; dorsomesial tubercle row often present; carpal groove impression weak to strong, occasionally absent. *Pereopod 2* variable in length (but approximate with OCL).

Sternal keel anterior lateral process shallow to deep, distally pointed to rounded; anterior margins of processes shorter, equal to, or longer than posterior margins; processes often meeting centrally. Median keel narrow to well-rounded; median keel mesial ridge weak to strong. Posterior process shallow to deep, narrow to broad; anterior margins of processes straight to curved, shorter, equal to posterior margins; processes often meeting centrally. Male genitalia consisting of a large, nonlobed, fleshy, semi-cylindrical outgrowth on mesial side of coxa.

Uropod endopod variable in width; mesial spine either single terminal or single terminal with one or more mesolateral spines; telson short.

***Spinastacoides inermis* Clark, 1939**

(Figure 5.18)

Etymology: inermis; Clark does not provide the etymology of this name, however; Latin adjective; *inermis*, unarmed, without weapons or defenceless.

Parastacoides inermis Clark, 1939: 126, Riek, 1967: 1002, Riek, 1969: 892, Lake & Newcombe, 1975: 197.

Parastacoides sternalis Riek, 1967: 1002, Riek, 1969: 892.

Parastacoides tasmanicus inermis Clark, Sumner, 1978: 819, Swain et al., 1977:85, Richardson & Swain, 1980:475, Richardson, 1983:239, Richardson & Horwitz, 1988:93, Horwitz, 1989: 30.

IS, Hansen and Richardson 1999a

Material examined

Syntype Male (NMV J889). 21.44 mm OCL, at 2850 feet on Adamsons Peak, Tas., J. Thwaites.

Other material examined. ♂ (ZUT HI3) 16.14 mm OCL, in buttongrass heath on slope at plain west of Scotts Peak Road near Harlequin Hill, Tas., 8112: 475 425, 4.03.1983, AMMR, RS. ♂ (ZUT HI12) 15.86 mm OCL, same data as HI3. ♂ (ZUT HI16) 21.64 mm OCL, same data as HI3. ♂ (ZUT LF5) 14.40 mm OCL, under rocks and moss at lake margin at Lake Fortuna, Western Arthurs Range, Tas., 8111: 372 248, 16.11.1982, AMMR, RS, DS. ♂ (ZUT LF15) 12.56 mm OCL, same data as LF5. ♂ (ZUT LF18) 15.30 mm OCL, same data as LF5. ♂ (ZUT LJ1a) 18.76 mm OCL, in thin soil over glacial till under open sedge heath at Lake Judd track, Tas., 8111: 490 370, 22.02.1987, AMMR, IG. ♂ (ZUT LJ1b) 18.24 mm OCL, same data as LJ1a. ♂ (ZUT LJ5) 12.96 mm OCL, in shallow burrow in peat under sedge heath at ridge east of Lake Judd track, Tas., 8112: 495 371, 22.01.1987, AMMR, IG. ♀ (ZUT HI6) 18.72 mm OCL, same data as HI3. ♀ (ZUT HI10) 17.50 mm OCL, same data as HI3. ♀ (ZUT HI15) 17.62 mm OCL, same data as HI3. ♀ (ZUT LF8) 17.24 mm OCL, same data as LF5. ♀ (ZUT LF11) 20.40 mm OCL, same data as LF5. ♀ (ZUT LF17) 20.66 mm OCL, same data as LF5. ♀ (ZUT LJ2) 17.94 mm OCL, same

data as LJ1a. ♀ (ZUT LJ3) 19.62 mm OCL, same data as LJ5. ♀ (ZUT LJ4) 22.84 mm OCL, same data as LJ5.

Diagnosis

Uropod endopod bearing single, terminal spine; great chelae adductor boss weakly developed.

Description:

Antennal scale lateral margin usually straight, sometimes curved; spine strong, forming lateral margin; distal margin usually entire, sometimes excavate or curved. *Rostrum* length 0.74-0.12 OCL, width 0.79-1.3 rostral length; rostral profile usually concave, sometimes straight in cross-section; rostral dorsal carina margin angled, apex acute; rostral lateral profile anteriorly depressed, distolaterally blunt. *Eye* 0.06-0.09 OCL; posterior margin of orbit usually entire, rarely notched; suborbital angle deeply curved to truncate. *Mandible* dentition formula usually 8-3, rarely 7-3 or 10-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes usually partially divided, sometimes fully divided, tubercles range small, clustered to large, discreet, distal margin usually curved, rarely straight.

Cephalothorax length 0.76-0.82 OCL. *Carapace* width 0.48-0.53 OCL, depth 0.55-0.63 OCL; dorsolateral bosses 0.3-0.63 OCL from eye orbit, positioned 0.59-0.72 carapace width on carapace; cervical groove shallow to deep, usually without notch when viewed dorsally, lateral setae present.

Great chelae length 1.08-0.63 OCL, width 0.37-0.53 chelae length, depth 0.52-0.6 chelae width; chelae ventral ridge extending proximal of cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.51-0.56 chelae length, depth 0.23-0.34 dactyl length, prodopus length 0.34-0.48 chelae length, depth 0.31-0.55 propodus length; dactyl and propodus tips directly opposed. Carpus length 1.1-1.37 carpus width, depth 0.73-0.96 carpus length, width 1.15-1.43 carpus depth; 4-6 dorsal tubercles forming distinct row;

dorsomesial tubercle row usually present; carpal groove absent to well-developed. *Pereopod 2* length 0.82-0.96 OCL, chelae 0.33-0.37 pereopod length.

Sternal keel anterior lateral process usually shallow and distally pointed; anterior and posterior margins of processes equal in length; processes usually meeting centrally. Median keel intermediate to well-rounded, never narrow; mesial ridge usually well-developed. Posterior process usually shallow and narrow, occasionally deep and broad; anterior margins of processes curved, longer than posterior margins of processes, processes meeting or not meeting centrally.

Uropod endopodite bearing single, terminal mesial spine; endopodite width 0.56-0.69 endopodite length; telson length 0.29-0.39 OCL.

Syntype Male

Antennal scale lateral margin straight; spine strong, produced from lateral margin; distal margin entire. *Rostrum* length 0.9 OCL, width 1.04 rostrum length; rostral profile straight in cross-section; rostral dorsal carina margin angled, apex acute; rostral lateral profile anteriorly depressed, distolaterally blunt. *Eye* 0.06 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.67 OCL. *Carapace* width 0.49 OCL, depth 0.57 OCL; dorsolateral bosses 0.32 carapace length from eye orbit, position on carapace 0.62 carapace width; cervical groove shallow, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 0.85 OCL, width 0.48 chelae length, depth 0.67 chelae width; chelae ventral ridge extending proximal of cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.53 chelae length, depth 0.3 dactyl length; prodopus length 0.37 chelae length, depth 0.5

propodus length; tips directly opposed distally. Carpus length 1.2 carpus width, depth 0.83 carpus length, width 1.43 carpus depth; 6 dorsal tubercles forming distinct row; dorsomesial tubercle row absent; carpal groove well-developed. *Pereopod 2* length 1.15 OCL, chelae 0.28 pereopod length.

Sternal keel anterior lateral process shallow, distally pointed; anterior and posterior margins of processes equal in length; processes meeting centrally. Median keel well-rounded; mesial ridge well-developed. Posterior process shallow, broad; anterior margins of processes curved, longer than posterior margins of processes, processes meeting centrally.

Uropod endopodite bearing single, terminal mesial spine; endopodite width 0.62 endopodite length; telson length 0.34 OCL.

Morphological Variation

This species can be distinguished from all other species of *Spinastacoides* by the presence of a single, terminal spine on the uropod exopodite combined with a weakly developed adductor boss on the great chelae, creating a distinctive "dish-shaped" palm.

As is the case with other *Spinastacoides* species, this species displays a degree of within-species morphological plasticity. Much of this variation can be related to geographical locality. Specimens of this species from the Harlequin Hill population (HI above) can be readily differentiated from those of other localities by: (1) the usually curved lateral and distal margins of the antennal scale, (2) the rostral dorsal profile, which is usually straight in cross-section, (3) the eye, which tends to be smaller, (4) the sagittiform, anteromedian lobe of the epistome, which is usually short and wide; and the posterolateral processes, which are fully divided, bearing small, clustered tubercles, (5) the weak development of the median keel and mesial ridge of the sternal keel, and (6) the narrow and shallow posterior processes of the sternal keel which meet centrally.

The mandible in *S. inermis* exhibits an unusual degree of variation; the number of corneous denticles varies from seven to ten, however the third denticle is always the largest. The usual combination was seen in the Syntype.

Whilst the cervical groove varies in depth, shallow grooves tend to be notched dorsally. The great chelae corresponded to the Syntype in specimens from the Lake Fortuna locality, elsewhere it tended to be more robust.

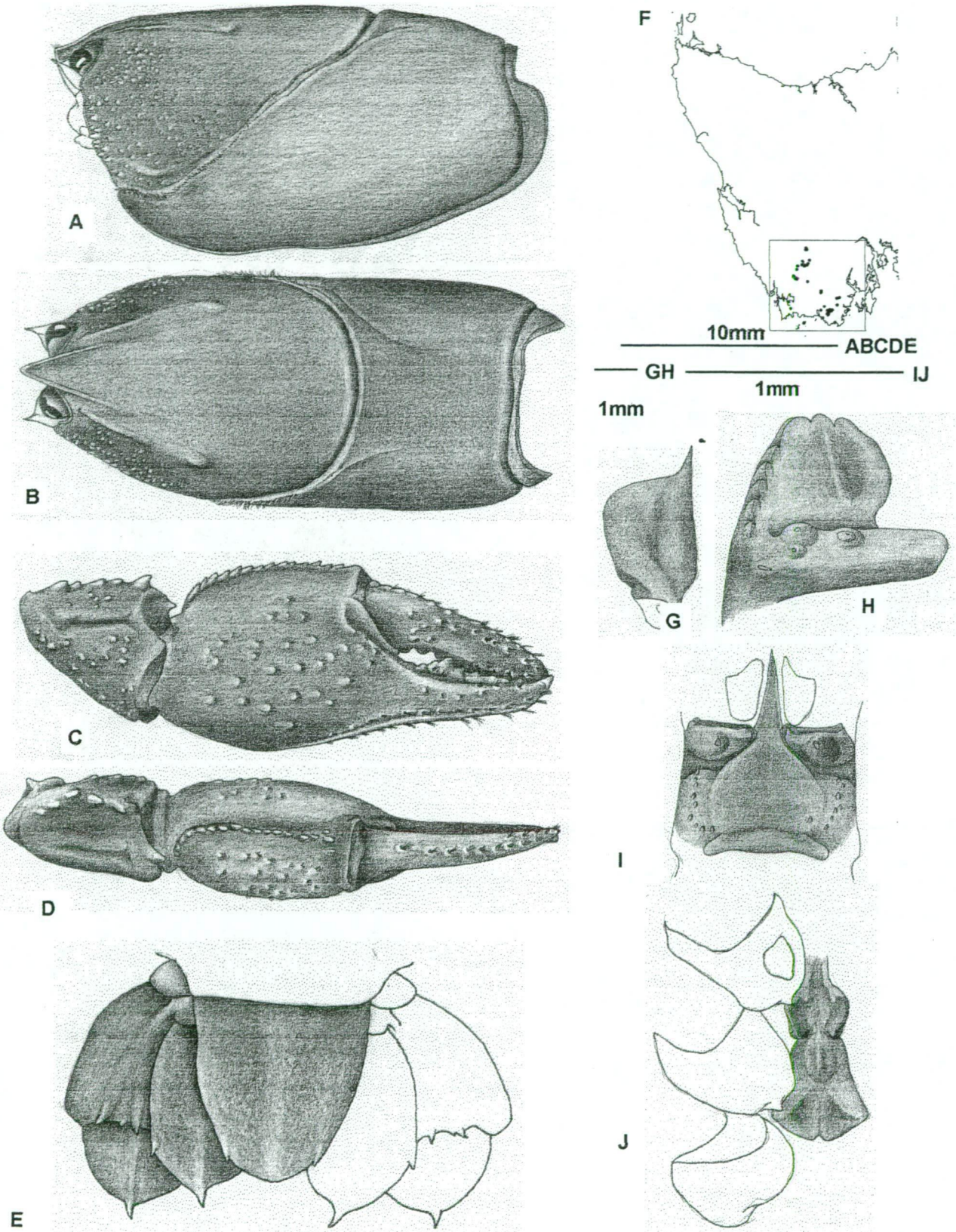


Figure 5.18. Spinastacoides inermis*: A-J from LJ4.*

***Spinastacoides insignis* Clark, 1939**

(Figure 5.19)

Parastacoides insignis Clark, 1939:126, Riek, 1967: 1000, Riek, 1972:371

Parastacoides tasmanicus insignis Clark; Sumner, 1978: 820; Swain *et al.*, 1977:85, Richardson & Swain, 1980:31, Horwitz, 1989:30.

I, Hansen and Richardson 1999a

Etymology: insignis; Clark does not provide the etymology of this name, however; Latin adjective; *insignis*, mark or emblem, eminent, distinguished or outstanding.

Material examined

Syntype Male (NMV J899) 22 mm OCL, in buttongrass at New Harbour, Tas., 27.01.1926 (C. King).

Other material examined

♂ (ZUT BCF5) 23.30 mm OCL, in peat under buttongrass at Bramble Cove, Port Davey, Tas., 8011: 184 36, 1.02.1982, RBM. ♂ (ZUT BCF6) 19.04 mm OCL, same data as BCF5. ♂ (ZUT BCF7) 17.12 mm OCL, same data as BCF5. ♂ (ZUT CRD1) 22.36 mm OCL, on dry slope in open heath at Crossing River at Port Davey track crossing, 8111: 323 268, 1.11.1981, AMMR, RS, DAR, RH. ♂ (ZUT CRD11) 17.64 mm OCL, same data as CRD1. ♂ (ZUT T14) 20.30 mm OCL, in buttongrass heath slope at Olga Valley (Line 7), Tas., 8012: 26 545, 7.02.1978, AMMR, RS, TF. ♂ (ZUT R22) 19.78 mm OCL, same data as T14. ♂ (ZUT AR16) 14.18 mm OCL, same data as T14. ♀ (ZUT BCF2) 22.62 mm OCL, same data as BCF5. ♀ (ZUT BCF4) 17.14 mm OCL, same data as BCF5. ♀ (ZUT BCF9) same data as BCF5. ♀ (ZUT CRD6) 23.40 mm OCL, same data as CRD1. ♀ (ZUT CRD18) 23.60 mm OCL, same data as CRD1. ♀ (ZUT AR24) 17.42 mm OCL, same data as T14. ♀ (ZUT R20) 19.90 mm OCL, same data as T14. ♀ (ZUT T16) 22.72 mm OCL, same data as T14. IS (ZUT CRD3) 19.30 mm OCL, same data as CRD1. IS (ZUT CRD15) 23.34 mm OCL, same data as CRD1.

Diagnosis

Uropod endopodite bearing one terminal and two or more distomesial spines.

Description:

Antennal scale lateral margin straight; spine strong, forming lateral margin; distal margin usually straight or curved. *Rostrum* length 0.05-0.13 OCL, width 0.07-0.18 rostral length; rostral profile usually concave, often straight in cross-section; rostral dorsal carina margin straight, apex usually rounded, sometimes acute; rostral lateral profile usually anteriorly depressed, but may be straight or upturned; rostrum usually distolaterally blunt, rarely acute. *Eye* 0.06-0.1 OCL; posterior margin of orbit usually entire, rarely notched; suborbital angle deeply curved to truncate. *Mandible* dentition formula 7-3 or 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes usually partially divided, occasionally fully divided, tubercles small, clustered to large, discrete, distal margin usually straight.

Cephalothorax length 0.65-0.82 OCL. *Carapace* width 0.5-0.54 OCL, depth 0.56-0.66 carapace width; dorsolateral bosses 0.33-0.39 OCL from eye orbit, at 0.6-0.72 carapace width; cervical groove deep to very deep, never shallow, deeply rounded U dorsally, occasionally notched, lateral setae present.

Great chelae length 0.81-1.05 OCL, width 0.44-0.59 chelae length, palm depth 0.56-0.96 chelae width; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss usually well-developed. Dactyl length 0.19-0.41 chelae length, depth 0.26-0.4 dactyl length; propodus length 0.32-0.44 chelae length, depth 0.48-0.65 propodus length; dactyl and propodus usually crossing distally, occasionally directly opposed. Carpus length 1-1.27 carpus width, depth 0.79-1 carpus length, width 1.06-1.54 carpus depth; 4-6 dorsal tubercles forming distinct row; dorsomesial tubercle row usually absent; carpal groove weak to well-developed. *Pereopod 2* length 0.84-0.94 OCL, chelae 0.34-0.37 pereopod length.

Sternal keel anterior lateral process usually shallow, distally pointed; anterior margins of processes shorter than or equal to posterior margins; processes not meeting centrally. Median keel well-rounded; mesial ridge well-developed. Posterior

process shallow, narrow; anterior margins of processes curved, shorter than posterior margins; processes meeting, or not meeting, centrally.

Uropod endopodite bearing single, terminal medial spine and two or more distomesial spines; exopod proximal segment intermediate in length; endopod width 0.56-0.71 endopod length; telson length 0.31-0.37 OCL.

Syntype Male

Antennal scale lateral margin straight; spine strong, forming lateral margin; distal margin entire. *Rostrum* length 0.11 OCL, width 0.8 rostral length; rostral profile straight in cross-section; rostral dorsal carina margin straight, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.07 OCL; posterior margin of orbit entire; suborbital angle truncate. *Mandible* dentition formula 7-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles large, discrete, distal margin straight.

Cephalothorax length 0.69 OCL. *Carapace* width 0.53 OCL, depth 0.64 carapace width; dorsolateral bosses 0.33 OCL from eye orbit, lateral placement 0.68 carapace width; cervical groove very deep, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 1.01 OCL, width 0.46 chelae length, palm depth 0.59 chelae width; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss weakly developed. Dactyl length 0.54 chelae length, depth 0.29 dactyl length; propodus length 0.41 chelae length, depth 0.6 propodus length; dactyl and propodus directly opposed distally, tips not overlapping. *Carpus* length 1.2 carpus depth, depth 0.84 carpus width, width 1.26 carpus depth; 6 dorsal tubercles forming distinct row; dorsomesial tubercle row present; carpal groove well-developed. *Pereopod 2* length 0.99 OCL, chelae 0.35 pereopod length.

Sternal keel anterior lateral processes shallow, distally pointed; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel well-rounded; mesial ridge well-developed. Posterior process shallow, narrow;

anterior margins of processes curved, shorter than posterior margins; processes meeting centrally.

Uropod endopodite bearing single, terminal medial spine and two or more distomesial spines; exopod proximal segment intermediate in length; endopod width 0.5 endopod length; telson length 0.35 OCL.

Morphological Variation

S. insignis is morphologically variable and much of this variation can be attributed to its wide geographic range. As in *S. inermis*, some populations within its range may be morphologically distinct; however, one character is stable and diagnostic: the multiple terminal spines on the uropod endopodite.

Specimens from the Olga Valley (T, R and AR above) are readily recognisable: (1) the antennal spine distal margin is entire, (2) the rostral profile is straight in cross-section profile, (3) the posterolateral processes of the epistome are all fully divided, bearing large, discrete tubercles, (4) the posterior processes of the sternal keel tend to have straight anterior margins, equal in length to the posterior margins and not meeting centrally.

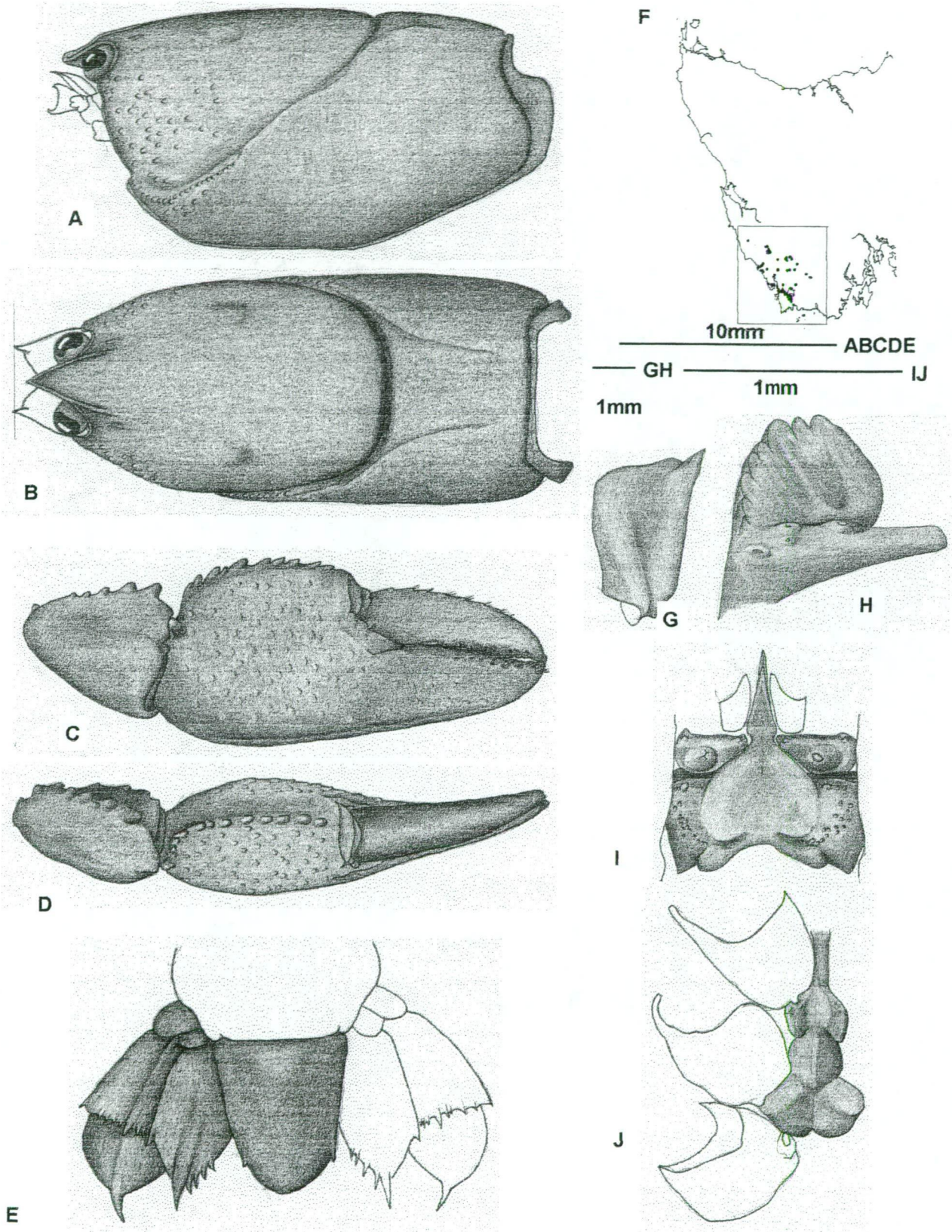


Figure 5.19. Spinastacoides insignis*; A-J from BCF6.*

***Spinastacoides catinipalmus*, sp. nov.**

(Figure 5.20)

WCI, Hansen and Richardson 1999a

Etymology: catinipalma; Latin compound noun; "dished palm", from *catinus*, a bowl and *palmus*, the palm of the hand, referring to the concave lateral surface of the propodus of the great chelae.

Holotype Female (ZUT WLI14) 21.00 mm OCL, under moss and logs in sandy shallow creek bed at Indiana Creek, 500 m up road from Warners Landing, Lower Gordon River, 8012: 926 863, 23.11.1984, AMMR, PHJH, RBM, RH, DS.
Allotype Male (ZUT WLI26) 19.28 mm OCL, in shallow burrow under moss and logs in rainforest seepage, 8012: 926 863, 1.05.1988, AMMR, RBM, RH, PH.

Other material examined

♂ (ZUT MMC1) 20.98 mm OCL, in burrow marginal to creek and under debris in creek at Dacrydium Creek, Mount McCall, Tas., 8013: 948 94, 15.02.1983, AMMR, RBM. ♂ (ZUT MMC10) 23.34 mm OCL, same data as MMC1. ♂ (ZUT MMC15) 25.64 mm OCL, same data as MMC1. ♂ (ZUT V1444) 17.16 mm OCL, in seepage draining into River Derwent, 2 km west of Wayatinah, Tas., 8113: 573 74, 9.01.1961, VVH, JLH. ♂ (ZUT V1459) 20.54 mm OCL, same data as V1444 except 15.01.1970, JLH, ISW, JO. ♂ (ZUT V1460) 13.14 mm OCL, same data as V1459. ♂ (ZUT WLI9) 20.00 mm OCL, same data as Holotype. ♂ (ZUT WLI23) 20.90 mm OCL, same data as Holotype. ♀ (ZUT MMC7) 28.88 mm OCL, same data as MMC1. ♀ (ZUT MMC9) 22.94 mm OCL, same data as MMC1. ♀ (ZUT MMC16) 27.68 mm OCL, same data as MMC1. ♀ (ZUT V1440) 25.46 mm OCL, same data as V1444. ♀ (ZUT V1441) 24.00 mm OCL, same data as V1444. ♀ (ZUT V1443) 14.88 mm OCL, same data as V1444. ♀ (ZUT WLI21) 20.80 mm OCL, same data as Holotype. ♀ (ZUT WLI27) 26.34 mm OCL, same data as WLI26.

Diagnosis

Uropod endopod bearing single, terminal spine; great chelae adductor boss well developed.

Description:

Antennal scale lateral margin usually straight; spine weak to strong, not forming lateral margin; distal margin entire or excavate. *Rostrum* length 0.06-0.12 OCL, width 0.69-1.11; rostral profile usually concave to straight in cross-section; rostral dorsal carina straight or angled, apex usually acute; rostral lateral profile straight or anteriorly depressed, margin usually distolaterally acute. *Eye* 0.07-0.08 OCL; posterior margin of orbit usually entire; suborbital angle deeply curved to (rarely) truncate. *Mandible* dentition formula 8-3, 10-3, 10-4. *Epistome* sagittiform, anteromedian lobe usually long, narrow; posterolateral processes usually fully divided, tubercles usually small, clustered, distal margin usually curved.

Cephalothorax length 0.73-0.82 OCL. *Carapace* width 0.47-0.52 OCL, depth 0.52-0.65 OCL; dorsolateral bosses 0.31-0.37 OCL from eye orbit, lateral position on carapace 0.52-0.7 carapace width; cervical groove shallow to deep, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 0.84-1.11 OCL, width 0.43-0.51 chelae length, depth 0.53-0.59 chelae width; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.53-0.6 chelae length, depth 0.23-0.32 dactyl length, propodus length 0.41-0.48 chelae length, depth 0.39-0.55 propodus length, dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1.11-1.42 carpus width, depth 0.7-0.9 carpus length, width 1.06-1.29 carpus depth; 3-7 dorsal tubercles, usually not forming distinct row; dorsomesial tubercle row usually absent; carpus groove weak to well-developed. *Pereopod 2* length 0.86-0.99 OCL, chelae 0.33-0.36 pereopod length.

Sternal keel anterior lateral process shallow to deep, usually distally pointed; anterior margins of processes usually shorter than posterior margins; processes rarely meeting centrally. Median keel narrow to well-rounded; mesial ridge usually well-developed. Posterior process usually deep and narrow; anterior margins of processes curved,

anterior and posterior margins usually equal in length, processes usually meeting centrally.

Uropod endopodite bearing single, terminal mesial spine; endopod width 0.55-0.68 endopod length; telson length 0.31-0.36 OCL.

Holotype female

Antennal scale lateral margin straight; spine strong, not forming lateral margin; distal margin excavate. *Rostrum* length 0.09 OCL, width 1.09 rostrum width; rostral profile straight in cross-section; rostral dorsal carina straight, apex rounded; rostral lateral profile anteriorly depressed, margin distolaterally acute. *Eye* 0.06 OCL; posterior margin of orbit entire; suborbital angle deeply curved. *Mandible* dentition formula 8-3. *Epistome* sagittiform, anteromedian lobe long, narrow; posterolateral processes partially divided, tubercles small, clustered, distal margin curved.

Cephalothorax length 0.77 OCL. *Carapace* width 0.5 OCL, depth 0.57 OCL; dorsolateral bosses 0.36 OCL from eye orbit, lateral position on carapace 0.52 carapace width; cervical groove shallow, deeply rounded U in dorsal view, lateral setae present.

Great chelae length 1.00 OCL, width 0.47 chelae length, depth 0.55 chelae width; chelae ventral ridge extending proximal of propodus cutting surface; chelae lateral surface setose-tuberculate; chelae adductor boss strongly developed. Dactyl length 0.56 chelae length, depth 0.28 dactyl length, propodus length 0.45 chelae length, depth 0.45 propodus length, dactyl and propodus directly opposed distally, tips not overlapping. Carpus length 1,2 carpus width, depth 0.83 carpus length, width 1.21 carpus depth; 5 dorsal tubercles not forming distinct row; dorsomesial tubercle row absent; carpus groove weak. *Pereopod 2* length 0.9 OCL, chelae 0.36 pereopod length.

Sternal keel anterior lateral process deep, distally pointed; anterior margins of processes shorter than posterior margins; processes not meeting centrally. Median keel well-rounded; mesial ridge well-developed. Posterior process deep, narrow; anterior margins of processes curved, anterior and posterior margins equal in length, processes meeting centrally.

Uropod endopodite bearing single, terminal mesial spine; endopod width 0.63 endopod length; telson length 0.32 OCL.

Allotype male

Specimen as per Holotype except: *Antennal scale* distal margin entire. *Rostrum* dorsal carina margin angled, apex acute. *Eye* suborbital angle shallowly curved. *Epistome* sagittiform, anteromedian lobe short, wide. *Carapace* cervical groove deep. *Sternal keel* anterior lateral process anterior and posterior margins equal in length. Median keel narrow.

Morphological Variation

Specimens from the population at Wayatinah (V above) can be immediately distinguished morphologically; they exhibit a curved antennal scale lateral margin, and an acute dorsal carina which is often distolaterally blunt. The eye in the Wayatinah population is more variable in width; ranging from small through to large. The mandibular structure is uniquely variable. Most individuals showed a mandible bearing a combination of eight corneous denticles, the third of which was the largest (8-3); the Wayatinah population differed, two individuals showed an 10-3 combination and three had a 10-4 combination. There is a tendency for the carapace to be wider in the Wayatinah population; the sternal keel always shows narrow posterior processes.

1. The variability in mandibular configuration of this species is of particular interest; it is the only species exhibiting all combinations. The Indiana Creek and Dacrydium Creek populations all possess a mandible dentition formula of

8-3. However, as mentioned above, the Wayatinah population is quite variable. This variability is not present in any other specie of *Spinastacoides*.

The shape of the great chelae is distinctive, being comparatively slender but with a distinctively scooped palm created by a well-developed adductor boss.

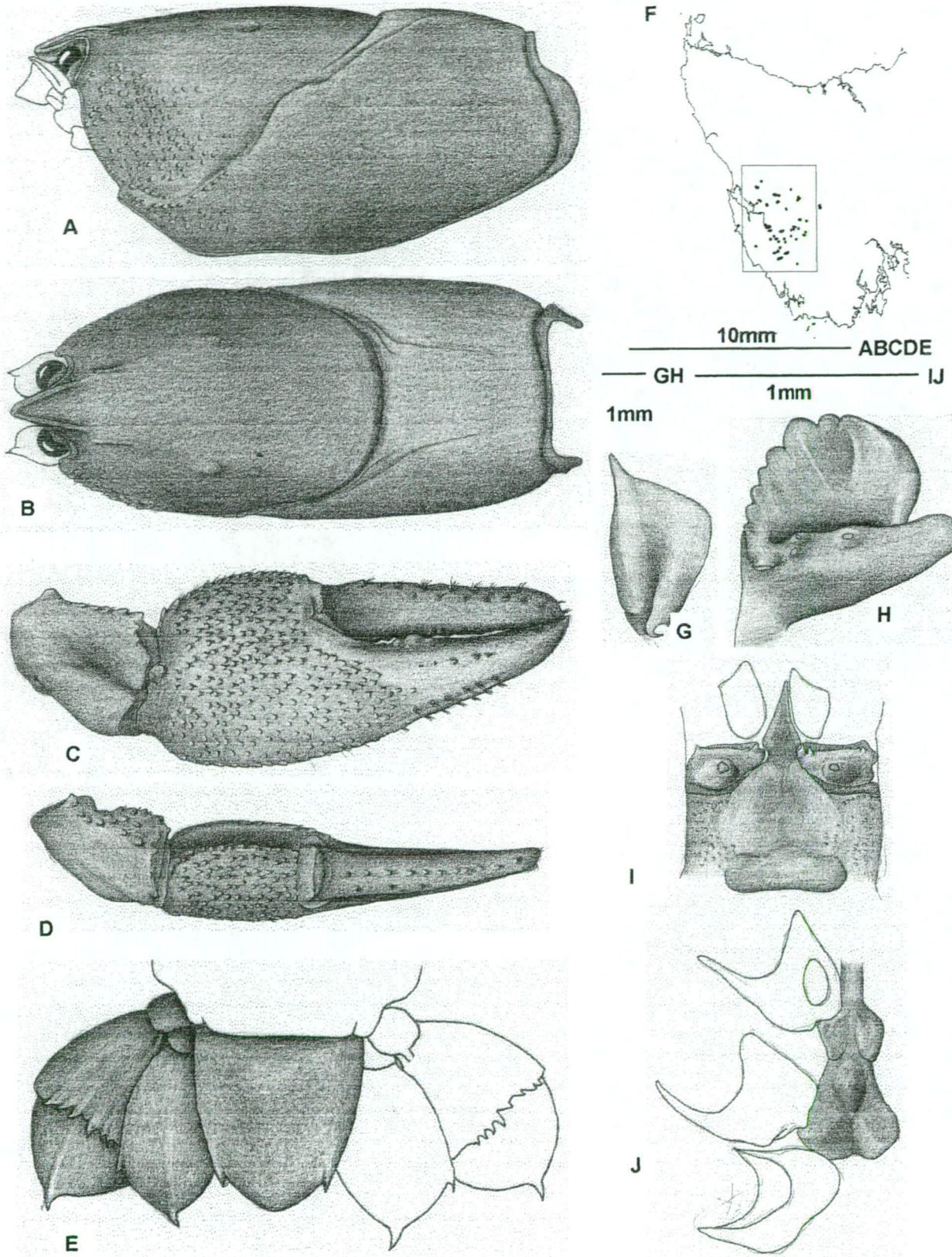


Figure 5.20. Spinastacoides catinipalma*; A-J from WLI14.*

6. The Biogeography of *Ombrastacoides* gen. nov. and *Spinastacoides* gen. nov.

Abstract

The School of Zoology at the University of Tasmania has a substantial collection of *Parastacoides* specimens (more than 1600 specimens). From these data, the species distributions of two endemic Tasmanian freshwater crayfish genera, *Ombrastacoides* gen. nov. and *Spinastacoides* gen. nov., were mapped. The sizes and shapes of the distributions varied; some of the *Ombrastacoides* species showed very restricted ranges: *O. denisoni, O. dissitus, O. parvicaudatus, O. professorum* and *O. ingressus*, while others had large and mostly exclusive ranges. *Spinastacoides* species all have relatively large ranges, ranging in a step-wise fashion, in southern Tasmania; a small contact zone exists along the margins where distributions meet.

While the majority of species' distributions lie within the boundaries of the Western Tasmania World Heritage Area, and they are consequently well protected, conservation concerns are justified for some species as their ranges lie either mostly, or in some cases entirely, outside the boundaries of the World Heritage Area, leaving them vulnerable. For example, the entire range of *O. denisoni* lies in an area designated for future forestry operations, and *O. parvicaudatus* may be extinct due to inundation of the known distribution area by waters from Lake Burbury, a hydro-electric lake.

Sympatric distributions of species both within and between the two genera were common, however areas of sympatry appear to be confined to narrow contact zones where distributions meet.

Most *Ombrastacoides* and *Spinastacoides* species appear to be capable of exploiting a wide variety of habitat types. It appears that ecological factors such as vegetation type, temperature and altitude, are not major determinants in the distribution of the species within each genus. Adequate rainfall and a low evaporation rate appear to be the major determinants restricting the overall distribution of the two genera, the low rainfall and high evaporation rate east of the 1000mm isohyet creating a barrier to further eastward range expansion for the two genera. However, rainfall and

evaporation rates do not appear to influence the distribution of the species within the genera. It appears that historical factors have played an important role in the present distribution patterns of species.

6.1 The Present Distribution of *Ombrastacoides* and *Spinastacoides* Species

Introduction

Riek (1972) suggested that parastacids, the Southern Hemisphere freshwater crayfish, could be divided into two structural and ecological groups. The separation was mostly based on the plane and movement of the dactyl of the great chelae along with some characteristics of the cervical and branchiocardiac grooves; in the *Engaeus* group the plane of the chelae was described as vertical, whereas in the *Euastacus* group the plane was more or less horizontal. *Parastacoides* (divided here into the new genera *Ombrastacoides* and *Spinastacoides*) did not fit well into either group, however Riek suggested that *Parastacoides* showed greater affinity to the *Euastacus* group. According to this classification, the *Engaeus* group are strong burrowers whereas the *Euastacus* group mainly inhabit streams and lakes and rarely need to dig deep burrows. This ecological classification does not apply well to either *Ombrastacoides* or *Spinastacoides*; they are strong burrowers (some burrows may be two metres deep (pers. observ.)) and, with the exception of one or two species, they are not strongly associated with permanent water bodies. The study by Crandall *et al.* (1999) into the molecular phylogenetic relationships of Australian and New Zealand crayfish does not support Riek's ecological division; however, like Riek, they suggest a sister group relationship between the genus *Parastacoides* and the New Zealand *Paranephrops*.

Documenting the distribution of species is important, not only because of the ecological information contained in the distribution, for example on such habitat requirements as food availability, but also because distribution, when coupled with phylogenetic information, may provide clues to the role of geography in the mode of speciation and consequent range change (Barraclough and Vogler 2000). At the broad scale, it is known that the geographic distribution of the genus *Parastacoides* (now *Ombrastacoides* and *Spinastacoides*) (Figure 6.1.1) occupies much of the western half of Tasmania. However, distribution can be defined in several ways: for

example, by the straightforward geographical range of a species or group of species (Lawrence 1996); or by type, for example, sympatric distribution; or by habitat, such as freshwater. The first section of this Chapter is concerned mainly with the geographic distribution, while the second section discusses influences likely to have had an effect on the geographical distribution of the two genera and species within the genera.

When considered on a smaller scale, the terminology used to describe distributions often refers to the spatial arrangement of species' distributions. Lawrence (1996) ascribes allopatry to species that have separate or mutually exclusive areas of geographical distribution, while species inhabiting the same or overlapping distributions are sympatric, and species whose distribution meet or have very narrow contact zones are described as having parapatric distributions. Lincoln *et al.* (1998) describe parapatric populations as having contiguous but not overlapping distributions. Lincoln's definitions are used throughout this Chapter, that is, species are defined as sympatric if they have any overlap in distribution, and species are described as parapatric if no record of distributional overlap exists.

In this Chapter, distributions of genera and species are mapped, then the elements that are likely to influence the distribution of firstly the two genera, but then also the species within these genera, will be discussed. Particular attention was given to the effects of vegetation pattern, soils and climate on distribution.

Materials and Methods

The School of Zoology, University of Tasmania, has a collection of over 1600 *Parastacoides* specimens, which can now be divided into approximately 900 *Ombrastacoides* and more than 600 *Spinastacoides* specimens. Data for each specimen, including map co-ordinates, are stored in Excel files. These map co-ordinates were used to map known distributions of the species of the two genera using the desktop mapping program MapInfo (1999). Specimens at putative distribution boundaries were examined for diagnostic characters (but were not fully scored as were the specimens included in the taxonomic study) and boundaries fully established. Tables 6.1.1 and 6.1.2 indicate the number of populations and number of specimens used to plot the distribution of each species of the genera *Ombrastacoides*

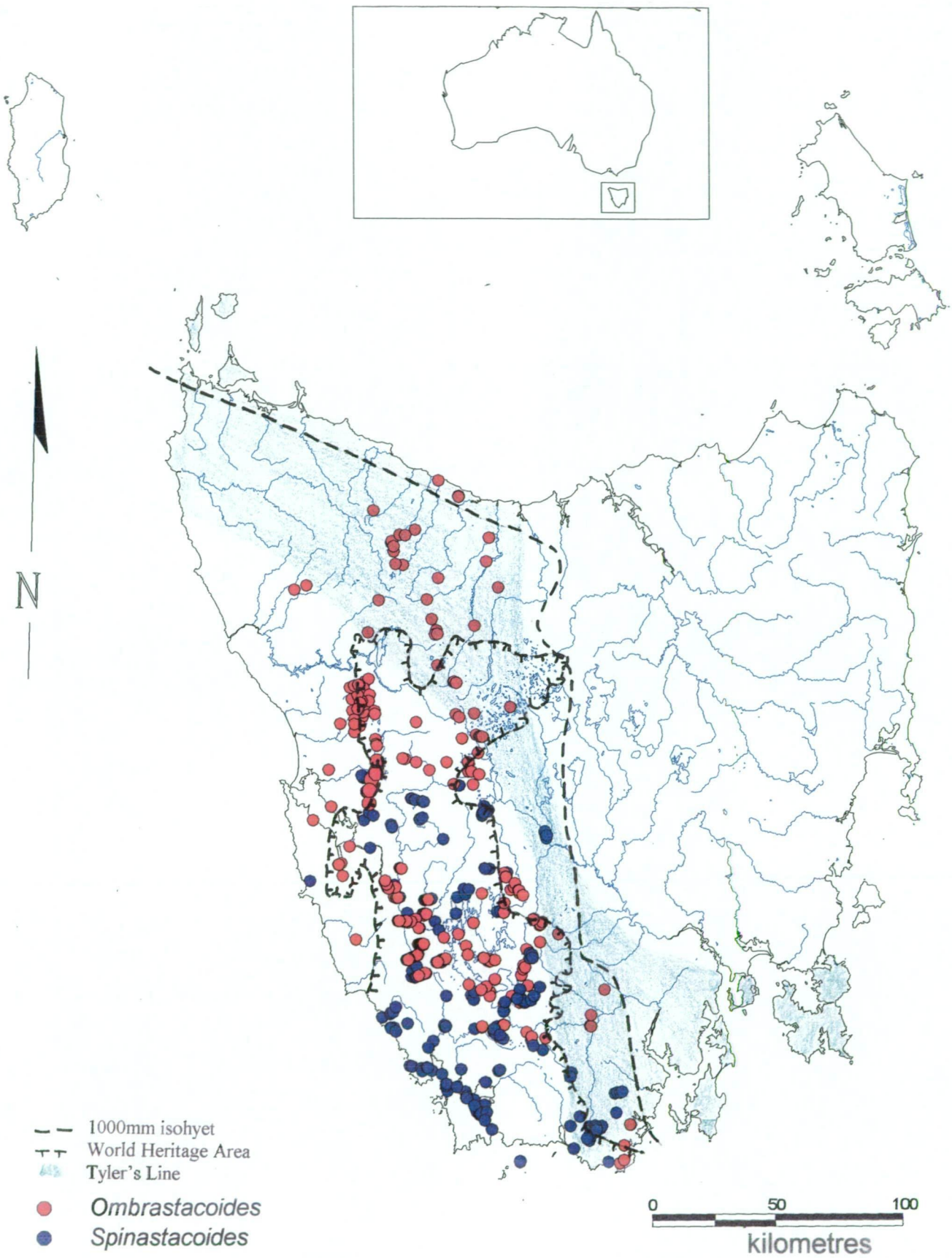


Figure 6.1.1. *Distribution of* Ombrastacoides *and* Spinastacoides. *Also indicated are the 1000mm isohyet, Tyler's Line and the boundaries of the World Heritage area.*

collected. Minimum and maximum distribution polygons were plotted (Table 6.1.3). Figure 6.1.2 illustrates the difference between these two methods. Maximum convex polygons were plotted over each distribution, assuming crayfish exist between these known collection points. Minimum distribution polygons were plotted; a polygon was drawn encircling the minimum area possible encompassing all known collection points.

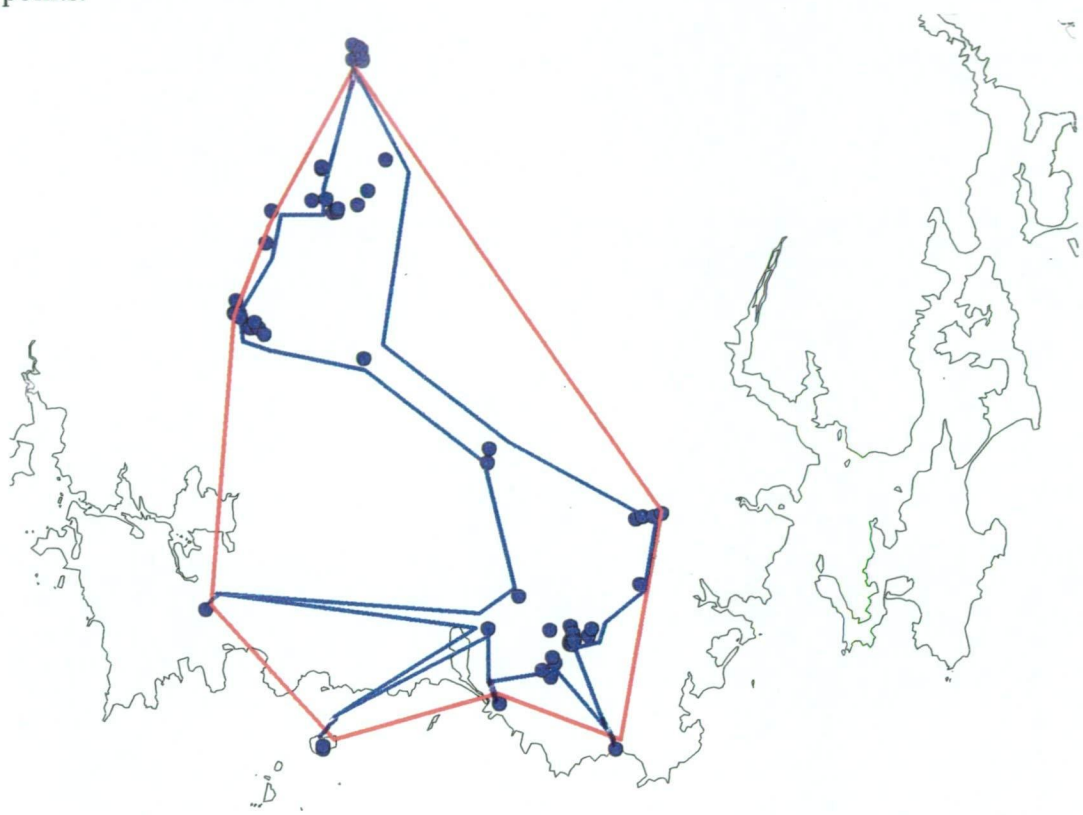


Figure 6.1.2. *Figure illustrating the difference between a maximum distribution polygon (indicated by the red outline) and a minimum distribution polygon (indicated by the blue outline). Blue dots represent known populations.*

Areas and perimeters of the maximum and minimum distribution polygons of each genus and species were calculated, using the functions in MapInfo. Distributions were also plotted as presences or absences in each square of a 10km^2 grid. Sympatric contact zones (hereafter referred to as SCZs) are areas where populations of more than one species occur in sympatry, while in the rest of each species' range is allopatric. SCZs were plotted using a modified maximum distribution polygon; obvious areas where crayfish would not be present, such as Macquarie Harbour were ignored, however specimens were generally assumed to be present between known collection localities.

Table 6.1.1. *Species, populations and number used to plot the distribution of species of the genus* Ombrastacoides.

Species	Populations	Number
asperrimanus	2	50
brevirostris	122	225
decemdentatus	20	78
denisoni	1	4
dissitus	5	21
huonensis	42	177
ingressus	2	25
leptomerus	90	293
parvicaudatus	3	5
professorum	9	37
pulcher	6	17

Table 6.1.2. *Species, population and number used to plot the distribution of species of the genus* Spinastacoides.

Species	Populations	Number
catinipalma	88	315
inermis	53	155
insignis	83	266

Results

Figure 7.1.3 shows the principal localities mentioned in the following text. The distributions of the two genera are shown in Figure 6.1.1 as one dot for each population. The map clearly illustrates the areas where the genera overlap in their distributions.

Ombrastacoides

Figure 6.1.4 indicates the distribution of the *Ombrastacoides* species. Each symbol represents one population and may therefore represent more than one individual (see Table 6.1.1). Species occur throughout the western half of the State, with the exception of the far northwest corner and the southwest corner. Four *Ombrastacoides* species occur in the King River Valley in the Lake Burbury region: *O. parvicaudatus, O. professorum, O. brevirostris, O. leptomerus.* Two species, *O. parvicaudatus* and *O. professorum*, are endemic to this region. One other species, *O. ingressus*, is restricted to an area close to this, centred on Victoria Pass. Two species occur in areas isolated from other *Ombrastacoides* species: *O. denisoni* in one small

region of the Little Denison River catchment in the south east, and *O. dissitus* in the far south east, east of the Mt. La Perouse, Precipitous Bluff mountain ranges. Only one species, *O. leptomerus*, occurs north of 42°S (or north of Lake Burbury).

The ranges of two species have been severely affected by hydro-electric lakes. *Ombrastacoides parvicaudatus* had an extremely restricted distribution in the King River valley around the region now inundated by Lake Burbury, and may well be extinct. *Ombrastacoides pulcher* is restricted to the northern shores of Lake Pedder. The range of this species appears to have been significantly reduced by the flooding of Lake Pedder.

Spinastacoides

Figure 6.1.5 illustrates the distribution of the three *Spinastacoides* species; each symbol represents one population and may therefore represent more than one individual (see Table 6.1.2). *Spinastacoides* species occur throughout the western half of the State south of 42°S. *Spinastacoides catinipalma* has the most northerly and the largest distribution of the three species. The distribution of *S. insignis* lies to the south of *S. catinipalma*, with a small area of sympatry at the southern edge of *S. catinipalma's* range. The distribution of *S. inermis* lies south and east of *S. insignis*, with an area of sympatry at the eastern margin of *S. insignis'* range.

Sympatric Contact Zones

Table 6.1.3 gives details of sympatric species. Sites within two of these SCZs have been extensively studied: the Olga River Valley and Harlequin Hill areas. Richardson and Swain (1980) carried out an intensive study in an area of the Olga River Valley in which *O. brevirostris, S. catinipalmus* and *S. insignis* occur in sympatry, investigating many aspects including vegetation, landscape, slope, drainage, soil texture and burrow structure. The Harlequin Hill region has been used for several studies including a study into the pattern and persistence in the burrows of *O. huonensis* and *S. inermis* when occurring in sympatry (Richardson and Swain 1991), and a study into the interaction between crayfish burrows and vegetation (Wong 1991).

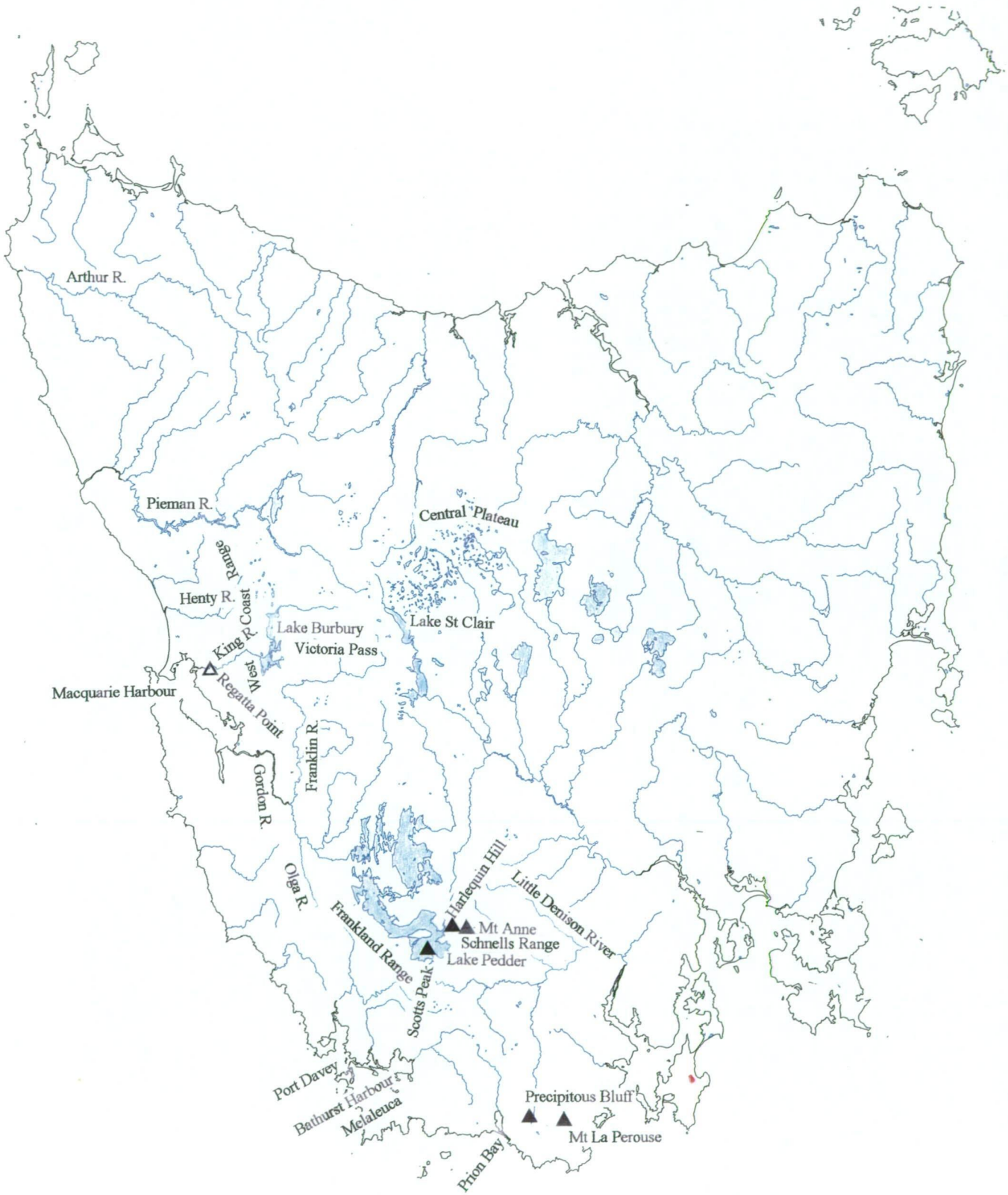


Figure 6.1.3. *Map indicating locality names used in text describing distributions.*

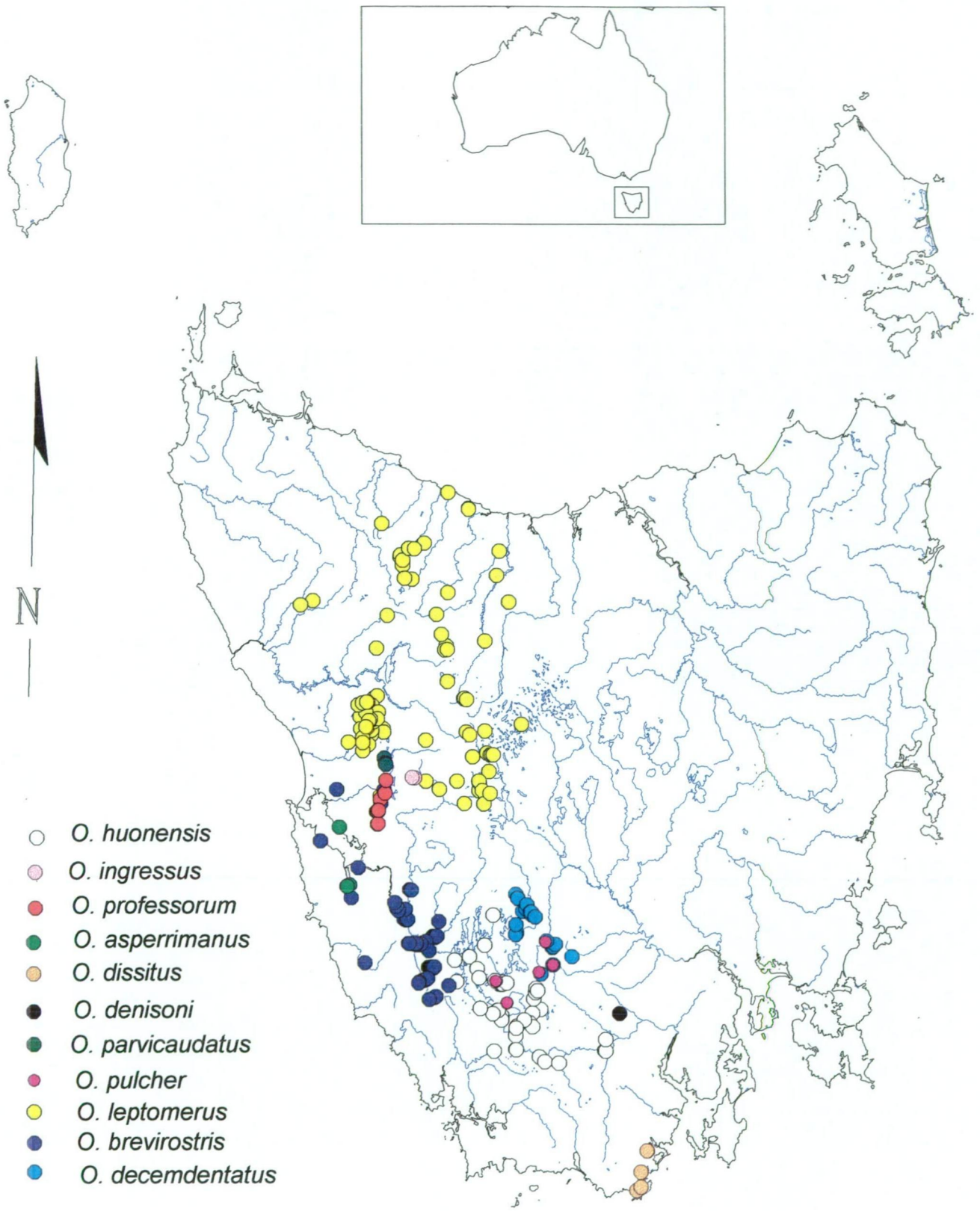


Figure 6.1.4. *Distribution of* Ombrastacoides *species in Tasmania.*

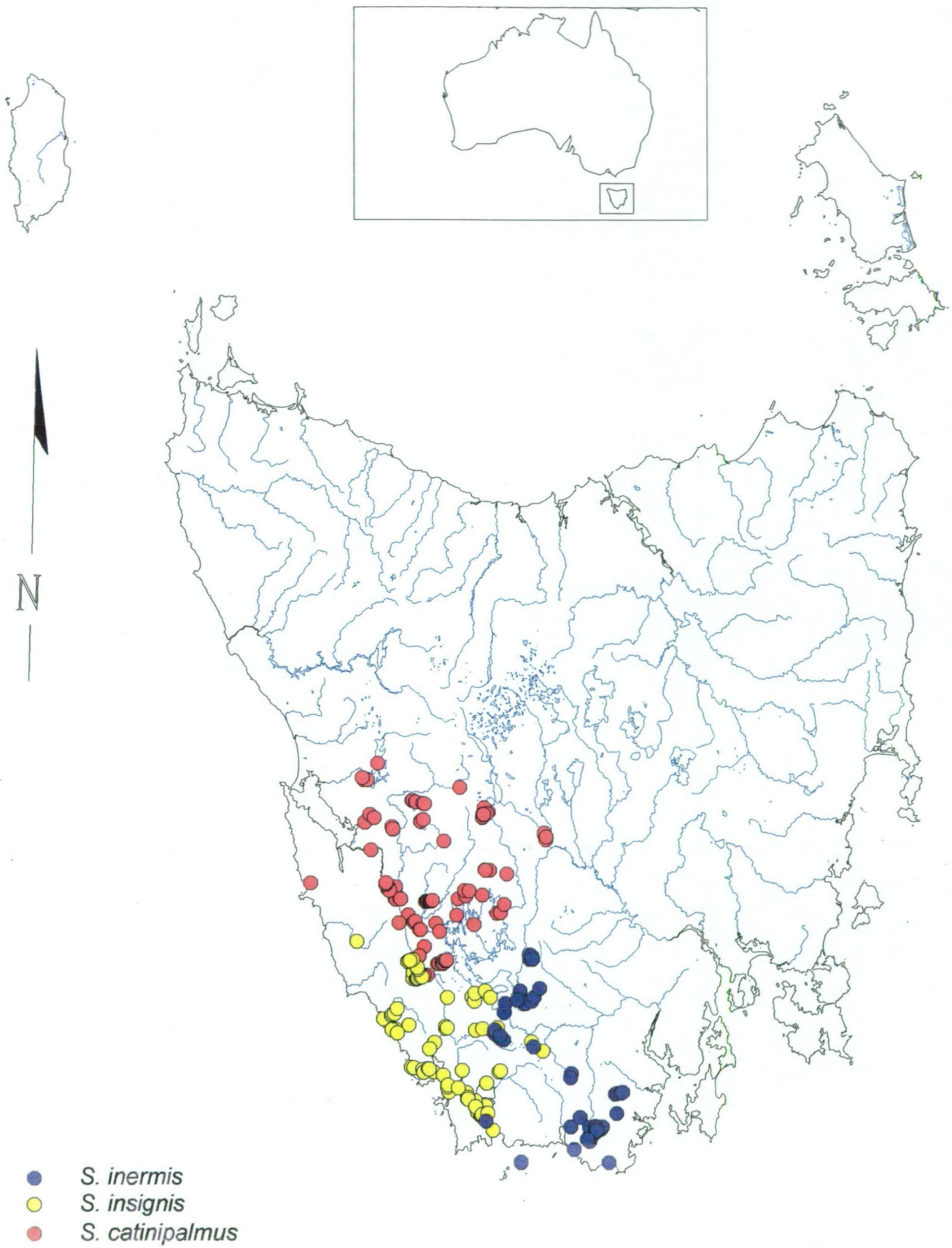


Figure 6.1.5. *Distribution of* Spinastacoides *species in Tasmania*

Table 6.1.3. *Matrix of sympatry between species of* Ombrastacoides and Spinastacoides. *Known sympatric occurrences are indicated by *; possible, but unrecorded sympatric occurrences are indicated by ?.*

Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14
S. insignis	1	-	*		*										
S. inermis	2		-	?							*	?	*	?	?
S. catinipalmus	3			-	*	*			?		?				
O. brevirostris	4				-	?	?			*					
O. leptomerus	5					-	?	?	?						
O. professorum	6						-								
O. parvicaudatus	7							-							
O. ingressus	8								-						
O. asperrimanus	9									-					
O. decemdentatus	10										-	?	*		
O. pulcher	11											-	?		
O. huonensis	12										*		-		
O. dissitus	13													-	
O. denisoni	14														-

Figure 6.1.6. illustrates SCZs along the boundaries of the distributions of each of the three *Spinastacoides* species and SCZs between a number of *Ombrastacoides* species. Table 6.1.4 summarises the SCZ areas between genera and between species.

Table 6.1.4. *Summary of SCZ sizes.*

SCZ taxa	Percent of taxon distribution	Area (km^2)	Width (km)	Length (km)
Ombrastacoides	31	6522	65.25	123.7
Spinastacoides	51			
O. decemdentatus	31	101	9.07	10.08
O. pulcher	44			
O. pulcher	20	45.49	9.49	10.6
O. huonensis	2.5			
O. leptomerus	1.3	108	13.39	15.36
O. brevirostris	4			
S. insignis	4	116	24.6	56.66
S. inermis	4			
S. insignis	5	135.6	12.8	12.9
S. catinipalmus	2			

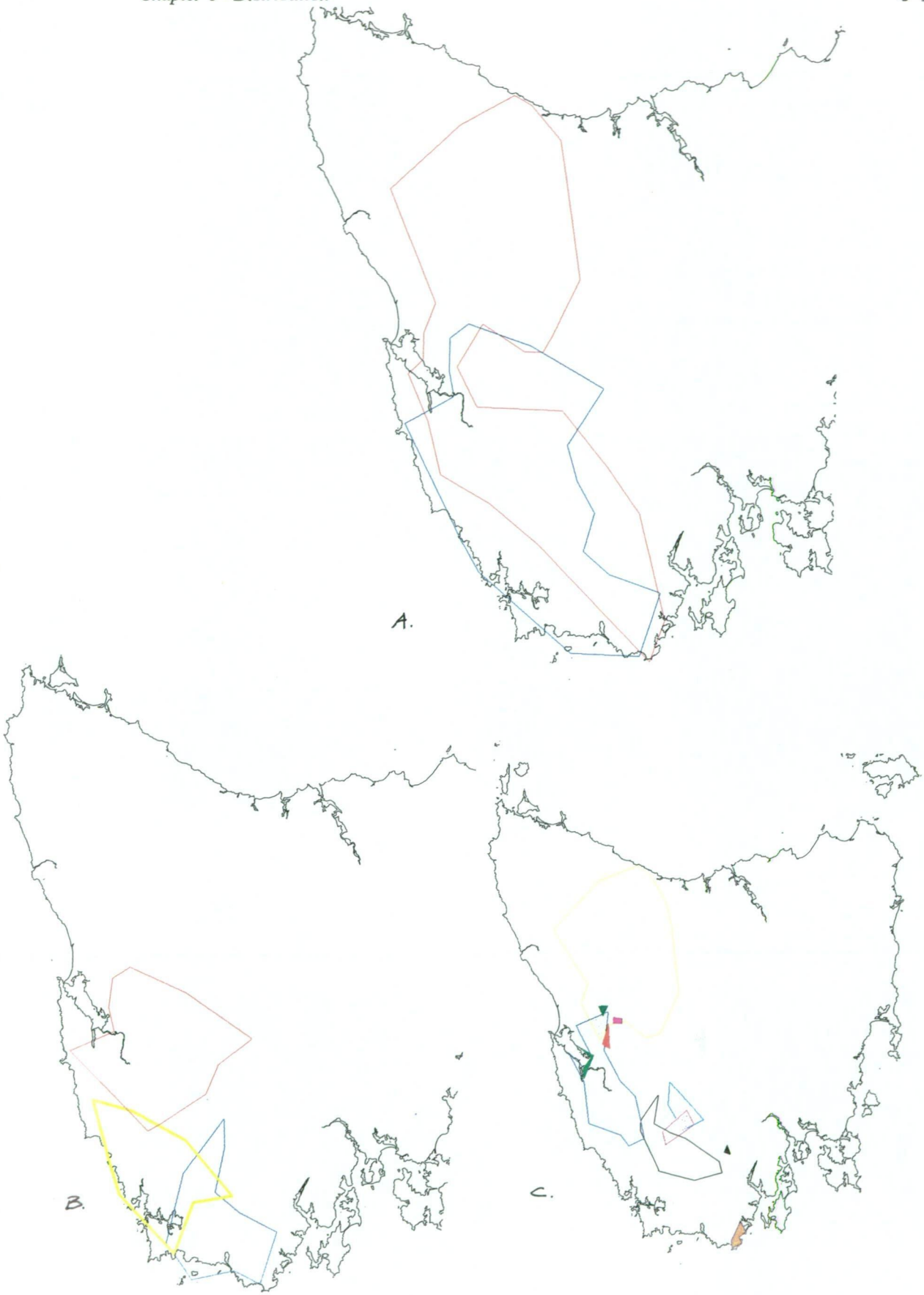


***Figure 6.1.6.**. A. The distributions of* Spinastacoides *species are indicated by polygons. Sympatric contact zones (SCZ) are the areas of overlap. B. The distributions of* Ombrastacoides *species are indicated by polygons. SCZs are the areas of overlap. C. The distribution of the genera* Spinastacoides *and* Ombrastacoides *are indicated by polygons. SCZs are the areas of overlap.*

Figure 6.1.7 shows the distributions of the two genera plotted on 10km^2 grids. Figure 6.1.8 ranks the ranges of *Ombrastacoides* and *Spinastacoides* species using ten-kilometre-square grid counts. Figure 6.1.9 shows the relationship between maximum and minimum polygon distribution areas for *Ombrastacoides* and *Spinastacoides* species in rank order. Although there appears to be some repetition, I feel it was important to indicate that whichever methodology one chooses to determine distribution size, the general trend in relation to size, was the same using all three methods (maximum distribution polygon, minimum distribution polygon and 10 km^2 grids), with the exception of *S. inermis* and *S. insignis*, where the ranks change between the maximum and minimum distribution polygons. The ranges of the genera and species within the genera varied significantly (see Table 6.1.4). The *Ombrastacoides* species are far more variable in their distributional ranges (6-3230 km^2) than are the *Spinastacoides* species (3310-5950 km^2). The average range of the *Spinastacoides* species (4190.7 km^2) is larger than that of *Ombrastacoides* species (1349.6 km^2), and only three *Ombrastacoides* species have ranges exceeding 500 km^2 (*O. leptomerus*, *O. brevirostris* and *O. huonensis*). All *Spinastacoides* species have a range in excess of 3000 km^2, whereas only two *Ombrastacoides* have a range in excess of 3000 km^2 (*O. leptomerus* and *O. brevirostris*).

Table 6.1.4. *Range size data for genera and species.*

Taxa	Maximum polygon area (km^2)	Maximum polygon perimeter (km)	Minimum polygon area (km^2)	Minimum polygon perimeter (km)	Number of 10km^2 squares
O. asperrimanus	17.89	49.54	1.72	17.66	2
O. brevirostris	2733.00	228.30	830.10	381.3	15
O. decemdentatus	326.40	82.80	90.65	106.00	7
O. denisoni	0.10	0.02	0.10	0.02	1
O. dissitus	15.74	32.94	15.74	32.94	2
O. huonensis	1863.00	179.50	612.3	280.00	14
O. ingressus	0.47	3.29	0.47	3.29	1
O. leptomerus	8072.00	344	3958	633.4	37
O. parvicaudatus	0.10	5.76	0.10	5.76	1
O. professorum	19.89	36.14	4.04	36.80	2
O. pulcher	227.50	69.38	46.00	81.70	5
Ombrastacoides	20850.00	661.50	7490	1155	75
S. catinipalmus	5708.00	288.40	2516.00	517.50	36
S. inermis	3087.00	226.9	1076.00	329.40	25
S. insignis	2781.00	229.00	1474.00	391.10	18
Spinastacoides	12770.00	452.60	7214	730.90	79

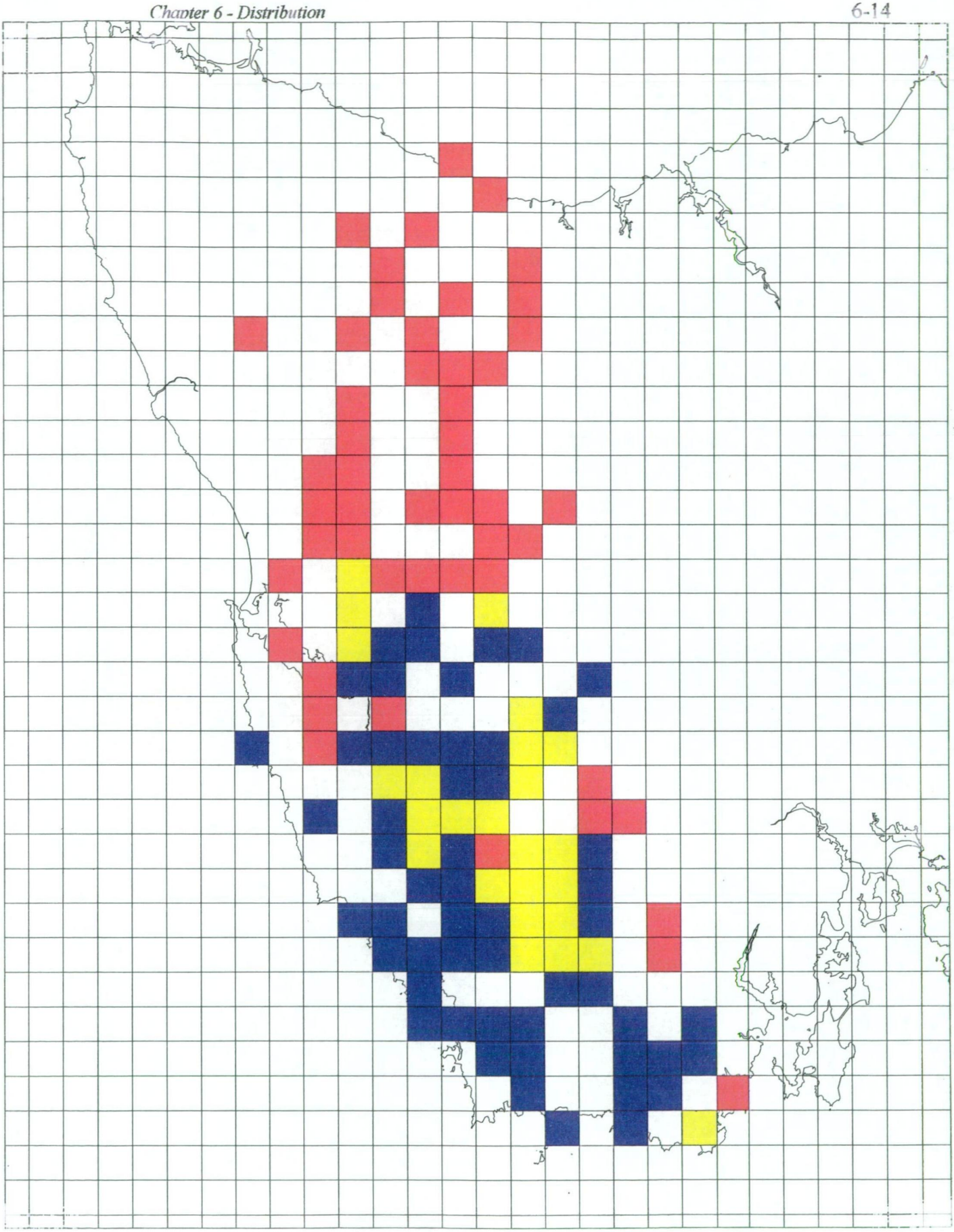


Figure 6.1.7. *Figure indicating the presence/absence of the two genera in 10 km² grids.* Ombrastacoides *species are indicated in red,* Spinastacoides *species are indicated in blue, and grids which contain both genera are indicated in yellow.*

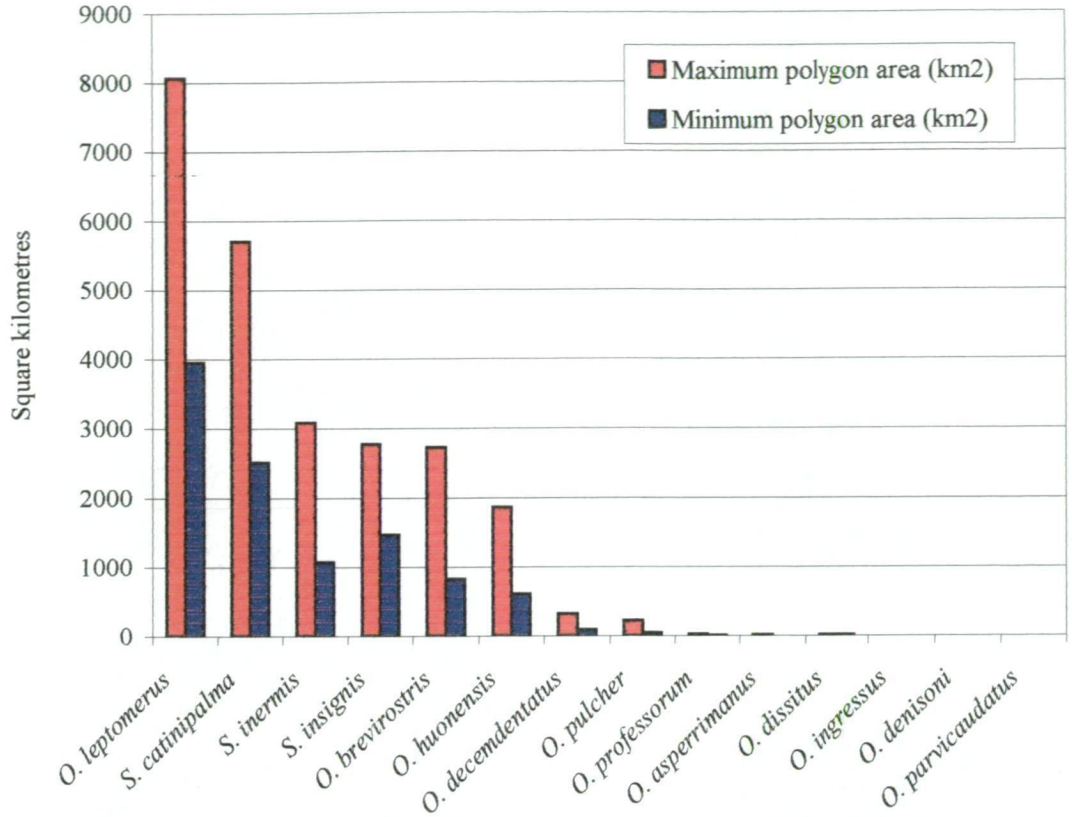


Figure 6.1.8. *Ranked ranges of maximum and minimum distribution polygon areas for* Ombrastacoides *and* Spinastacoides *species*

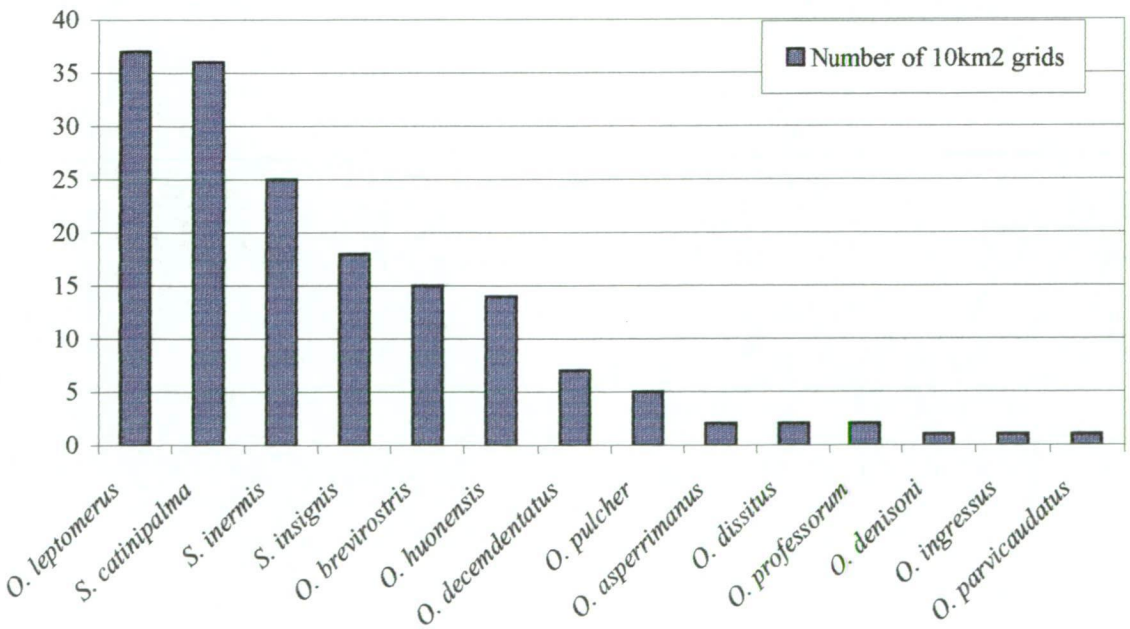


Figure 6.1.9. *Ranked ranges of* Ombrastacoides *and* Spinastacoides *species ranges using number of ten-metre-square grid presence counts*

Discussion

Both genera, *Ombrastacoides* and *Spinastacoides*, are endemic to Tasmania. Their distribution generally lies to the west of the 1000mm isohyet and the faunal break known as Tyler's Line (Figure 6.1.1). Tyler (1981) describes "*a line passing through the Derwent Bridge pub ... a natural geologic, climatic, edaphic and vegetational watershed*". He noted changes in the fauna, particularly west of the line, where possums become darker, and butterflies change colour and become new sub-species; "*west of the line the water retained the ionic character of their westerly oceans, acidified by plant remains till bicarbonate goes, so starved of calcium that local crayfish reserve it for the most essential parts of the skeleton*". This faunal break corresponds with sharp changes in environmental gradients, for example it approximates well to the 1000mm isohyet. Shiel *et al.* (1989) noted that water in lakes to the east of Tyler's Line are less humic, even alkaline, and that the lakes reflect geological and climatic differences between the two regions. They also commented on the demarcation of two distinct rotifer assemblages, separated into eastern and western communities by this faunal break. Jordan (1995) suggests that Tasmania can be divided into two biogeographic provinces by the 1000mm isohyet, with the western area exhibiting a relatively high diversity of conifer species, as well as other species groups. Mesibov (1994) suggests that faunal breaks, such as Tyler's Line, have the potential to provide historical zoogeographic information, particularly information on the evolution of invertebrates associated with the faunal break. Faunal breaks such as Tyler's Line are generally associated with ecotones (Mesibov 1994), or zones where two ecosystems overlap (Lawrence 1995); these ecotones are capable of supporting fauna from both ecosystems, and sometimes fauna associated only with the ecotone. Ecotones at faunal breaks potentially mark the distributional limit of habitat, but equally, for slowly dispersing fauna an ecotone may represent a dispersal barrier despite suitable habitat on the other side.

The area in which the two genera are distributed has remained relatively geomorphologically stable for some time (pers. comm. Prof J.B. Kirkpatrick, School of Geography and Environmental Studies, University of Tasmania). The occurrence of diamictites of possible glacial origin (Kiernan, 1985) hints that parts of the region occupied by *Ombrastacoides* and *Spinastacoides* species may have been glaciated as long ago as the Precambrian. There is evidence of Late Carboniferous continental ice sheets spreading across Tasmania from Gondwanan Antarctica, and of continuing ice

rafting into the Late Permian. The dolerite in regions of *Ombrastacoides* and *Spinastacoides* distribution is of Jurassic origin and most likely represents a response to the breakup of the Gondwanan supercontinent. The uplift and southeastwards tilting of the Central Plateau commenced approximately 65 Myr ago as Antarctica separated from Australia. Basalt eruptions in the northern parts of the range of *O. leptomerus* date from the Late Oligocene – Early Miocene. However, the deeply weathered mantles in Tasmania date from at least the Early Tertiary, and are compatible with a prolonged period of geologic and geomorphic stability in the mountains of western central Tasmania. Kiernan suggests that this landscape has remained essentially unchanged from the Early – Middle Tertiary until the Pleistocene glaciations. There is fossil evidence from Central Tasmania which points to this period as being warm and moist, supporting rainforest vegetation (Keirnan 1985). Tasmania is divided into two distinct structural provinces (Davies 1965); the western portion of Tasmania, more or less corresponding to the distribution of the two genera, is composed of intensely folded basement of quartzose sediments. The large rivers in this western fold structure province, such as the Arthur, Pieman, King and Gordon, which all contain populations of both genera, are ancient systems. These river systems pre-date the present folded mountain chains, and have cut deep gorges through the mountain chains as the land was uplifted (Jackson 1999b).

Several factors may control the distribution of an organism, for example habitat, diet and vagility, ie their dispersal capabilities. Horwitz (1986) suggests that certain assumptions can be made regarding the dispersal capabilities of freshwater crayfish. Species capable of occupying Type 2 burrows (burrows that are not connected to surface water, but are connected to the water-table (Horwitz and Richardson 1986)), as all *Ombrastacoides* and *Spinastacoides* are, appear less likely to disperse actively by swimming across areas covered by flood waters, and thereby moving long distances via permanent water bodies such as streams. It appears most likely, in adults at least, that individuals walk above ground when the humidity is sufficient to allow this (Horwitz *et al.* 1985). Thus their most likely method of dispersal is by self-propulsion, either in water bodies or terrestrially, and they should be considered as having very low vagility. In one area occupied by *Ombrastacoides huonensis* and *Spinastacoides inermis,* a ten year study in an area of 108 contiguous four square-metre quadrats (Richardson and Swain 1991) observed only one new burrow system.

They suggested that burrows persist beyond the life span of individual occupants; dispersing juveniles appear to occupy vacant burrow systems rather than starting new ones. Juveniles stay in the maternal burrow system for approximately one year after hatching and then disperse when the ground surrounding the burrow of the mother becomes saturated, the water table rises and some pools form (autumn and early winter) (Lake and Newcombe 1975).

There appear to be four major clusters of species' distributions within the genus *Ombrastacoides*: 1) a northern group containing the species *O. ingressus, O. leptomerus, O. parvicaudatus*, and *O. professorum*; 2) a western group comprising *O. asperrimanus* and *O. brevirostris*; 3) a central group with four species, *O. decemdentatus, O. denisoni, O. huonensis* and *O. pulcher*; and 4) a southern group consisting of only one species, *O. dissitus*. Group 1 contains closely related sister taxa, as does Group 3, however the relationships between taxa in the other two groups are not as clear cut (see Chapters 2 and 3.2). Whether these groups represent centres of speciation without much dispersal, or relictual remnants from more widespread ancestral species is difficult to ascertain. Certainly the distribution of *O. leptomerus* must have been severely disrupted by the Early Pleistocene glacial events (see below), however it is doubtful whether later glacial events had as much impact. However great that impact, the range of *O. leptomerus* on the Central Plateau certainly represents expansion since the last, and certainly from earlier, glacial events, and as such, provides some indication of the possible rate of dispersal of this species. Kiernan's (1990a) map of glaciation on the Tasmanian Central Highlands (see Figure 6.2.6) indicates that populations of *O. leptomerus* would need to disperse approximately 10-15 kilometres to reach some present localities that were covered by ice during the last glacial event. These regions would have been completely ice free approximately eight to ten thousand years ago (Jackson 1999b). This suggests a minimum expansion rate of a little over one kilometre per thousand years, or one metre per year. This seems reasonable, despite the low rate of burrow excavation noted by Richardson and Swain (1991) above. Their study area was saturated with burrows, and the rate of burrow excavation may be increased where new, unexploited habitat is encountered.

Horwitz (1986) suggests that sympatry between species of freshwater crayfish will rarely be complete, and this appears to be the case where species of *Spinastacoides* and/or *Ombrastacoides* come into contact. Richardson and Swain (1980) conducted a study into the habitat preferences of the then three sub-species of *Parastacoides* (*Parastacoides tasmanicus tasmanicus* (*Ombrastacoides brevirostris*), *P.t. insignis* (*Spinastacoides insignis*) and *P.t. inermis* (*S. catinipalmus*)) in the valleys of the Gordon River and its tributaries, and locally at a site in the Olga valley. They concluded that differences existed between the sub-species. *O. brevirostris* was typically found in wet heath, Melaleuca swamp or rainforest vegetation, in poorly-drained, flat valley floors. However, in this area, *O. brevirostris* was never found in creeks or seepages, and rarely found in dry heath or dry Melaleuca vegetation. *Spinastacoides catinipalmus* was found in two disjunct habitats in this study area: dry, well-drained heath or Melaleuca-covered slopes, or under rocks in small shallow streams in rainforest vegetation. *Spinastacoides insignis* was only observed in the south west of their study area. It overlapped with both *O. brevirostris* and *S. catinipalmus* in both wet and dry heaths, however it was absent from rainforest, and never found in creeks or seepages. Richardson and Swain suggested that competition for space between *O. brevirostris* and *S. catinipalmus* was a possibility, especially as extensions of habitat exploitation was evident in both species in the absence of the other, while there was a possibility of competition between *O. brevirostris* and *S. insignis*, as their ranges overlapped widely. However, they found it was difficult to ascertain whether the absence of *S. insignis* from rainforest was due to competition or habitat choice, or merely a reflection of the unavailability of the habitat within its range. Results from the study by Hansen and Richardson (1999a) suggest that competition may be the cause of the exclusion of species from habitats at the Olga Valley site. *O. brevirostris,* which was never found in creeks or seepages at Olga Valley, is in fact significantly associated with those habitats in the rest of its distribution.

Most *Ombrastacoides* species do not commonly display peripatry or sympatry. The three species with restricted ranges within the range of *O. leptomerus*: *O. professorum*, *O. parvicaudatus* and *O. ingressus*, have not yet been recorded in sympatry with *O. leptomerus* (pers. com. A. Richardson, School of Zoology, University of Tasmania). Molecular data (Chapters 2 and 3.2) suggest that the origins

of these species were ancient, and no hybridisation has occurred. It is not possible to state with certainty by what mechanism *O. leptomerus* is excluded from the areas occupied by these other species. However, vegetation patterns do not appear to be involved, as *O. leptomerus* is quite capable of existing in the vegetation types used by the other species (see Chapter 6.2 for details), suggesting that, some form of competitive exclusion may be occurring, of the type described by Letcher *et al.* (1994).

It appears that habitat partitioning, or competitive exclusion, occurs when species are in SCZs, and habitat extension is apparent in the rest of the species' ranges. The small-scale habitat partitioning found between sympatric species *O. brevirostris, S. insignis* and *S. catinipalmus* in the SCZ in the Olga Valley (Swain and Richardson 1980) and between *O. huonensis*, and *S. inermis* in the Harlequin Hill area (Richardson and Swain 1991), may also suggest that contact zones are not the result of contact due to recent dispersal from the last glacial refuges. This degree of sorting and mutual adaptation suggests that the contact is far older. All SCZs occur at the margins of distributions, and are relatively small compared to the distribution of each species concerned. I suggest that this may be because the competitive exclusion from some habitats occurring in the SCZs prevents large range expansion into territories already occupied by a species.

Spinastacoides species display a different pattern of distribution from those of *Ombrastacoides*: 1) all species have a distribution of similar size, 2) all distributions are relatively large, and 3) the boundaries of all distributions have SCZs. Overall, areas of sympatry occur only at the margins of species distributions. This could suggest that, in contrast to *Ombrastacoides* spp., these species' ranges are expanding, perhaps from ranges reduced by adverse conditions during the last glacial event, and the areas of contact are recent. If this were the case, then possible glacial refuges for the three *Spinastacoides* species are: 1) Macquarie Harbour/Gordon River (*S. catinipalmus*), 2) Port Davey/Bathurst Harbour (*S. insignis*), and 3) Prion Bay (*S. inermis*). However, these may not have been refugia during the most recent glacial events; rather, they may reflect refugia from earlier glacial events, and subsequent dispersion (see below). One of these possible glacial refuges, Macquarie Harbour/Gordon River, corresponds with a possible refugia for a group of

Ombrastacoides species: *O. leptomerus*, *O. brevirostris* and *O. asperrimanus*, *O. parvicaudatus*, *O. professor* and *O. ingressus.*

Ombrastacoides species do not present as simple a pattern as that displayed by *Spinastacoides* species. The parapatric distribution clusters may represent refugial patterns, ie, refuges in the Bassian plains, Pieman, Henty, King and Gordon Rivers systems for *O. leptomerus*, *O. brevirostris* and *O. asperrimanus*, *O. parvicaudatus*, *O. professor* and *O. ingressus*, and a refuge centred near Lake Pedder for *O. decemdentatus*, *O. pulcher* and *O. huonensis*, *O. dissitus* and *O. denisoni*. But the lack of SCZs between species groups suggests: 1) recent allopatric speciation, or 2) it may indicate that the present patterns are relicts from once more widespread distributions. Areas of discontinuity may represent evidence of former habitat continuity (Udvardy 1969), especially in the case of *O. dissitus* and *O. denisoni*, with their restricted, isolated distributions in the south east of Tasmania.

As mentioned above, the SCZs between *S. catinipalmus* and some *Ombrastacoides* species may be the result of a shared refugium around the Macquarie Harbour, Gordon River region. The SCZs for *S. inermis* and the central group of four *Ombrastacoides species*, *O. decemdentatus*, *O. denisoni*, *O. huonensis* and *O. pulcher*, may represent a recent contact as these groups have extended their ranges out of refugia.

In terms of species richness, the King River Valley, around the new Lake Burbury region, has not only more taxa, but more endemic taxa than anywhere else in the State. Examination of the area in more detail (Figure 6.1.3) suggests that it supports a complex of sympatric, allopatric and perhaps even parapatric distributions of up to six species, five from the genus *Ombrastacoides* (*O. leptomerus*, *O. parvicaudatus*, *O. professorum*, *O. brevirostris* and *O. ingressus*) and one from the genus *Spinastacoides* (*S. catinipalmus*). The distribution of *O. professorum* appears to be allopatric, despite being within the range of *O. leptomerus*. Searches designed specifically to find areas of sympatry between these species failed to find any (A. Richardson, pers. comm.). This may also be the case with *O. ingressus*, however, more detailed surveys along the margins of its range are required to determine whether its distribution is really allopatric. All recorded sites of *O. parvicaudatus*

have been inundated by Lake Burbury, a hydro-electric lake, and the species may now be extinct.

In summary, *Ombrastacoides* and *Spinastacoides* show different distribution patterns. *Spinastacoides* species all occupy adjacent ranges of similar size in the southwest of the state, separated from each by only a small SCZ. *Ombrastacoides* species have more varied range sizes, some very restricted. *Ombrastacoides* species occur over a larger portion of western Tasmania than *Spinastacoides* species, and occur further north than *Spinastacoides* species. *Ombrastacoides* species do not all have SCZs. Where SCZs occur between species of either genus, habitat partitioning based on soil drainage is apparent, and appears to be driven by competitive exclusion. The competitive exclusion occurring between species may be limiting range expansion. The ranges of some of species lie outside the World Heritage area, and conservation concerns are warranted.

6. 6.2 Distributional Influences and Habitat Requirements

Introduction

The previous section defined the geographic distribution of *Ombrastacoides* and *Spinastacoides* species; the species comprising these two genera are limited to particular areas of Tasmania, and their distributional limits are set by ecological or historical attributes, or a combination of these attributes. Few detailed studies of the habitat preferences of individual species of the two genera have been attempted; the function of this section is to explore habitat factors that may have influenced the geographical distribution.

The majority of the information has been obtained from the School of Zoology crayfish collection database. The records are not complete however, and the number of specimen records, and the detail contained within the record, varied widely between species. The quality of data taken from the collection labels also varies widely between species and some species' habitat categories were not common enough to allow a valid analysis. Further amalgamation of the classes could not be used to solve this problem (see Appendix E) as further amalgamation would lead to unsuitable associations. For example, whilst buttongrass and graminoid heath can be amalgamated, coastal vegetation does not readily fit into any other grouping. However, despite this, useful information can still be obtained by analysing the database, as the wide variety of habitat classes exploited by these crayfish can be examined and described.

Vegetation as habitat

The interaction between vegetation and animals, such as crayfish, is complex, but a few studies into this interaction have been attempted concentrating on *Ombrastacoides* or *Spinastacoides* species (see Richardson and Swain 1978, Wong 1991, Richardson and Wong 1995). In this section, I looked at the vegetation in which of *Ombrastacoides* and *Spinastacoides* species were found, as determined from records in the School of Zoology's crayfish collection, to establish whether preferences for different vegetation types exist between different species or genera. It complements an earlier preliminary study (Appendix E) that investigated a series of habitat parameters including vegetation, but this study concentrated on species

having complete records for more than 100 specimens. The records of the School of Zoology crustacean collection were examined for information on vegetation type at the site where each specimen was collected. The vegetation descriptions on the specimen labels were simplified into nine categories (see Table 6.2.1). As in Appendix E, Specht's (1979) definition of "graminoid heathland" as "heaths with shrubs and monocotyledons co-dominant" was used as a single definition of many of the vegetation descriptions used on specimen labels.

Table 6.2.1 *Vegetation categories described on specimen labels, and the simplified categories to which they have been allocated.*

Vegetation type recorded	Vegetation Categories
buttongrass, buttongrass/melaleuca, buttongrass/heath, buttongrass/ sedgeland, buttongrass/spregelia, heath, sedgeland/heath, leptospermum scrub, bauera, sedgeland, Ghania, astelia, glychenia, moorland, pineapple grass	graminoid heath (GH)
melaleuca, melaleuca/eucalypt, melaleuca/heath	melaleuca (ML)
rainforest, rainforest/buttongrass ecotone, King Billy	rainforest (RF)
wet sclerophyll, mixed forest, wet sclerophyll/heath, forest, thick scrub	wet sclerophyll (WS)
sphagnum, sphagnum/glychenia	sphagnum (SM)
moss, moss/tussocks, mossy banks, moss bed	moss (M)
alpine scrub, alpine forest, cushion plants	alpine vegetation (AV)
palustral turf, coastal scrub, coastal grass, reeds	coastal vegetation (CV)

Table 6.2.2 lists the number of records of the major vegetation types in which *Ombrastacoides* species were found. Table 6.2.3 lists the number of records of the major vegetation types for *Spinastacoides* species; explanation of the vegetation codes used are given in the Table captions. Note however, that an * merely represents no mention in the record, and does not imply absence from that vegetation type.

Despite the inability to perform valid statistical analysis on these data, trends were nevertheless apparent. As can be seen from the data in the Tables 6.2.2 and 6.2.3, most species inhabit a broad range of vegetation types, the exceptions being the species with very limited ranges: *O. ingressus*, *O. parvicaudatus*, *O. denisoni*, and *O. pulcher*. It is not possible to determine from the collection database whether their absence from a vegetation type is real, or whether it merely reflects their restricted distribution.

Table 6.2.2. *Vegetation recorded at the vicinity of* Ombrastacoides *burrows. GH=graminoid heath, ML=melaleuca, RF=rainforest, WS=wet sclerophyll, SM=sphagnum moss, M=moss, AV=alpine vegetation, CV=coastal vegetation. Note the symbol* represents no record, and does not imply absence from the vegetation type.*

Species	GH	ML	RF	WS	SM	M	AV	CV	Veg type given	Total specimens
O. brevirostris	72	40	93	4	*	*	*	*	209	225
O. leptomerus	75	*	16	4	10	*	14	*	119	295
O. asperrimanus	5	25	*	*	*	*	*	*	30	30
O. decemdentatus	16	1	*	*	*	*	*	*	17	78
O. dissitus	14	*	1	3	*	*	*	*	18	21
O. ingressus	3	*	*	*	*	*	*	*	3	25
O. huonensis	106	*	4	1	*	*	*	*	111	146
O. professorum	20	*	*	*	*	*	*	*	20	37
O. denisoni	*	4	*	*	*	*	*	*	4	4
O. parvicaudatus	*	*	*	*	*	*	*	*	0	6
O. pulcher	10	*	*	*	*	2	*	*	12	34
Total	321	70	114	12	10	2	14	0	543	901

Table 6.2.3. *Vegetation recorded at the vicinity of* Spinastacoides *burrows. GH=graminoid heath, ML=melaleuca, RF=rainforest, WS=wet sclerophyll, SM=sphagnum moss, M=moss, AV=alpine vegetation, CV=coastal vegetation. Note the symbol* represents no record, and does not imply absence from the vegetation type.*

Species	GH	ML	RF	WS	SM	M	AV	CV	Veg type given	Total specimens
catinipalmus	64	17	66	6	1	21	*	*	175	315
inermis	49	*	12	2	*	17	7	2	89	155
insignis	165	21	9	1	*	12	3	1	212	266
Total	268	38	87	9	1	50	10	3	476	736

Ombrastacoides denisoni is only found in one small stream drainage. The crayfish inhabit deep burrows in extremely dense melaleuca shrub on one side of the road and stream. The other side of the road and stream consists of mature rainforest; despite extensive searching, no crayfish burrows were found in the rainforest, however, several specimens were found in shallow burrows in the roadside ditch. It is not clear whether the vegetation type, the stream, or some other factor formed a barrier to further expansion in the past for this species, but it is clear that they are capable of utilising novel habitats, i.e. the roadside ditch. For the other restricted species

mentioned above, the records are incomplete, and it is not known whether all specimens in the collection came from similar vegetation. These species with restricted distributions generally do not share distributions with other crayfish species (see above), and competitive exclusion may be occurring. This competitive exclusion may be preventing these species from using a wider range of vegetation habitats, rather than a physiological inability.

The major vegetation types for species of both genera are the wet heaths (buttongrass, graminoid heaths and melaleuca) and rainforests (Figure 6.2.1). Differences noted between the graminoid heaths and rainforest types may be due to sampling bias; crayfish burrows are harder to find and more difficult to excavate in rainforest areas; burrow entrances are more cryptic and the crayfish often make use of large tree roots in the construction of their burrows (pers. observ., Richardson and Swain 1978).

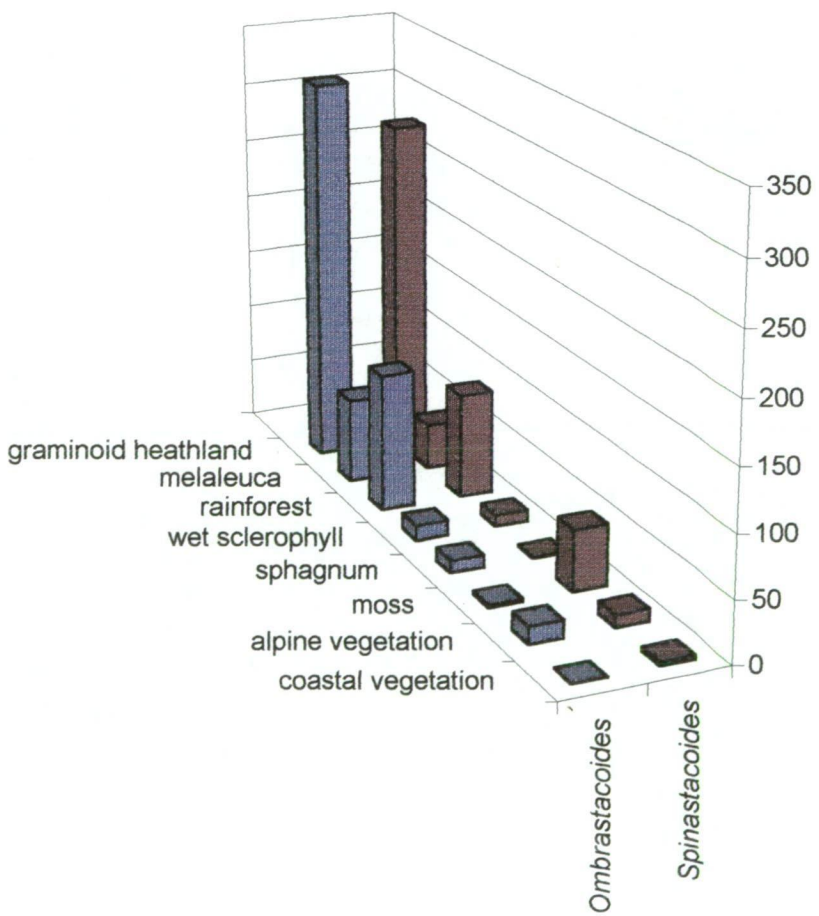


Figure 6.2.1. *Frequency with which* Ombrastacoides *and* Spinastacoides *species are associated with the vegetation variables.*

Wong's (1991) study into the interaction between *Parastacoides tasmanicus tasmanicus* (*O. huonensis*) and the buttongrass moorland at Harlequin Hill in central southern Tasmania suggested that the presence of crayfish burrows strongly correlated with areas of increased plant productivity. She was unable to conclude however, that the increases observed in plant diversity, cover and height, were the result of the presence of the crayfish burrows, or whether the crayfish and the plants were responding to the same abiotic factors, for example, drainage and water table level. Vegetation can reflect a number of environmental factors such as temperature, rainfall, soil type and drainage, so it is not always possible to tell which factor, or combination of factors, is driving habitat choice in invertebrates such as crayfish.

Habitat choice by crayfish might reflect differences in the soil habitat, to which the vegetation is also responding, which in turn could be related to physiological tolerances (eg to desiccation). Richardson and Swain (1978) suggest that two species in their study on habitat partitioning in the Olga Valley, *P.t. tasmanicus* (*O. brevirostris*) and *P.t. inermis* (*S. catinipalmus*), may be employing the same physiological adaptation for two different environmental stressors; *O. brevirostris* avoids hypoxia in poorly drained buttongrass-dominated peats by reducing metabolic demands, while *S. catinipalmus* avoids desiccation in dry heaths by the same adaptation.

In summary, nearly all *Ombrastacoides* and *Spinastacoides* species are capable of surviving in a wide range of vegetation types. The only species unlikely to be found in a wide range of vegetation types are those with very restricted ranges, and it is not clear from the data whether they are capable of using a wider range. Where a species with a restricted distribution has a restricted vegetation habitat usage, it is not clear what mechanism excludes it from other vegetation habitats. Crayfish with widespread distributions appear capable of exploiting all vegetation types within their distribution, and there is no evidence to suggest that any of the widespread species is incapable of utilising, or specialising in, any vegetation type.

Substrate as Habitat

The data for substrate are not as comprehensive as those on vegetation type (see Table 6.2.4). However, it is again possible to conclude that these crayfish are capable of exploiting a wide variety of substrate types (see Tables 6.2.5. and 6.2.6).

Table 6.2.7 details the geology types mentioned in the School of Zoology records as being used by the crayfish. Tables 6.2.8 and 6.2.9 provide a breakdown of this information by species from each genus. The crayfish are found on a variety of geologies, however the data are insufficient to suggest any real trends. Burrows are often in very acidic (pH<4) anaerobic peats; large populations of burrowing crayfish are not known to occupy this type of habitat any where else in the world (Growns and Richardson 1988).

Table 6.2.4. *Indicates the substrate types mentioned in the records, and the categories they have been assigned to.*

Substrate type in record	Amalgamated substrate category
peat, sandy peat, muck peat, organic ooze	peat
clay, organic clay, heavy clay	clay
sand, coastal, behind dunes, calcareous sand, white beach sand	sand
gravel, roadside gravel, glacial gravel, quartzite gravel, glacial till, fine gravel, muddy gravel, siliceous pebbles	gravel
mud, calcareous mud, fibrous black mud, black mud, black organic mud, light grey mud	mud
silt, wet black silt, chocolate silt	silt
soil, loam, organic soil, dry soil, soft slimy chocolate soil, rich brown soil, black soil	soil

Table 6.2.5. *Substrate type (with number of individuals) for each* Ombrastacoides *species, from information contained in the School of Zoology museum collection. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

species	peat	clay	sand	gravel	mud	silt	soil	Substrate given	Total specimens
O. brevirostris	56	19	14	5	24	16	17	151	225
O. leptomerus	23	2	*	13	*	*	1	39	295
O. asperrimanus	2	*	*	1	*	*	*	3	30
O.decemdentatus	1	*	*	*	*	*	*	1	78
O. dissitus	*	12	*	*	*	*	*	12	21
O. ingressus	*	*	*	*	*	*	*	0	25
O. huonensis	4	*	*	*	*	1	8	13	146
O. professorum	*	*	*	*	*	*	*	0	37
O. denisoni	4	*	*	*	*	*	*	4	4
O. parvicaudatus	*	*	*	*	*	*	*	0	6
O. pulcher	*	*	*	*	*	*	*	0	34
Total	90	33	14	19	24	17	26	223	901

Table 6.2.6. *Substrate type (with number of individuals) for each* Spinastacoides *species, from information contained in the School of Zoology museum collection. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

species	peat	clay	sand	gravel	mud	silt	soil	Substrate given	Total specimens
S. catinipalmus	26	15	15	11	5	*	5	77	315
S. inermis	11	2	1	*	2	*	*	16	155
S. insignis	55	*	2	4	47	*	5	113	266
Total	92	17	18	15	54	0	10	206	736

Table 6.2.7. *Indicates the geology types mentioned in the records, and the categories they have been assigned to.*

Geology type in record	Geology category
quartz sand, quartzite gravel, quartzite boulders	quartz
sandstone	sandstone
dolerite	dolerite
conglomerate	conglomerate
limestone	limestone
glacial gravel, glacial till, moraine	glacial deposit

Table 6.2.8. *Geology type (with number of individuals) for each* Ombrastacoides *species, from information contained in the School of Zoology museum collection. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

species	quartz	sand stone	dolerite	conglomerate	lime stone	glacial deposit	Geology given	Total specimens
O. brevirostris	7	*	*	*	*	*	7	225
O. leptomerus	5	*	3	*	*	6	14	295
O. asperrimanus	*	*	*	5	*	*	5	30
O. decemdentatus	*	*	*	2	*	*	2	78
O. dissitus	*	*	*	*	*	*	0	21
O. setosimerus	*	*	*	*	*	*	0	25
O. huonensis	*	*	*	*	*	*	0	146
O. professorum	*	*	*	*	*	*	0	37
O. denisoni	*	*	*	*	*	*	0	4
O. parvicaudatus	*	*	*	*	*	*	0	6
O. pulcher	*	*	*	*	*	*	0	34
Total	12	0	3	7	0	6	27	901

Table 6.2.9. *Geology type (with number of individuals) for each* Spinastacoides *species, from information contained in the School of Zoology museum collection. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

species	quartz	sand stone	dolerite	conglomerate	lime stone	glacial deposit	Geology given	Total specimens
S. catinipalmus	6	*	4	1	*	*	11	315
S. inermis	16	1	1	*	1	3	22	155
S. insignis	2	*	*	*	*	*	2	266
Total	24	1	5	1	1	3	35	736

Burrow Location as Habitat

Wong (1991) described distinct differences in the burrow locations of the two species at her two research sites. *Parastacoides tasmanicus tasmanicus* (*O. huonensis*) excavated burrows on waterlogged flats at Harlequin Hill, while *P.t insignis* (*S. insignis*) excavated burrows on drier slopes at Melaleuca. Whilst the Zoology records do suggest a trend for *Spinastacoides* species to inhabit drier slopes (30.2%), a substantial number of records indicate that *Ombrastacoides* species also inhabit drier slopes (8.1%). Approximately 27% of records stating the burrow location of *Ombrastacoides* species place them in plains, as opposed to only 1.6% for *Spinastacoides* species. *Ombrastacoides* species are found in creeks in 23% of the records, while *Spinastacoides* are found in creeks in 37% of cases. *Ombrastacoides* species occur in seepages in 25.2% of the records, while *Spinastacoides* are found in seepages in 14.5% of the records.

Table 6.2.9. *Indicates the burrow location types mentioned in the records, and the categories they have been assigned to.*

Location type in record	Location category
flat, plain, outer flood plain	plain (P)
slope, hillside, hill slope, dry slope	slope (S)
ridge, low ridge	ridge (R)
swamp, marsh	swamp (SW)
roadside ditch, road gravel	road (RD)
creek, creek edge, creek bank, inflow creek, top main creek, sandy bottom creek, small stream	creek (CR)
lake, lake edge, tarn, pool	lake (LK)
seepage, flood channel	seepage (SP)
under logs, fallen wood	under logs (UL)
track cording	track cording (TC)

Table 6.2.10. *Burrow locations in which* Ombrastacoides *species have been collected (from the School of Zoology, University of Tasmania crayfish collection). P = plain, S = slope, R = ridge, SW = swamp, RD = road, CR = creek, LK = lake, SP= seepage, UL = under logs, TC = under track cording. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

Species	P	S	R	SW	RD	CR	LK	SP	UL	TC	Location given	Total specimens
O. brevirostris	37	17	1	3	3	42	*	18	1	*	122	225
O. leptomerus	2	2	1	5	3	58	29	40	1	2	143	295
O. asperrimanus	*	1	*	*	1	*	*	28	*	*	30	30
O. decemdentatus	*	1	*	*	*	1	9	27	*	*	38	78
O. dissitus	*	*	*	*	*	1	*	1	*	2	4	21
O. ingressus	25	*	*	*	*	3	*	*	*	*	28	25
O. huonensis	68	19	*	1	*	6	1	9	1	*	105	146
O. professorum	*	*	*	6	*	*	*	*	*	*	6	37
O. denisoni	*	*	*	*	*	*	*	*	*	*	0	4
O. parvicaudatus	*	*	*	*	*	3	*	*	*	*	3	6
O. pulcher	*	*	*	15	*	*	*	2	*	*	17	34
Total	132	40	2	30	7	114	39	125	3	4	496	901

Table 6.2.11. *Burrow locations in which* Spinastacoides *species have been collected (from the School of Zoology, University of Tasmania crayfish collection). P = plain, S = slope, R = ridge, SW = swamp, RD = road, CR = creek, LK = lake, SP= seepage, UL = under logs, TC = under track cording. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

Species	P	S	R	SW	RD	CR	LK	SP	UL	TC	Location given	Total specimens
S. catinipalmus	*	*	*	*	*	95	12	31	*	*	138	315
S. inermis	*	22	*	2	*	5	16	11	2	1	59	155
S. insignis	5	74	4	3	*	18	*	4	*	*	108	266
Total	5	96	4	5	0	118	28	46	2	1	305	736

In summary, *Ombrastacoides* species tend to occur on plains, creeks and seepages, but are also located on drier slopes a substantial proportion of the time. *Spinastoacoides* species tend to be located on drier slopes, creeks and seepages, but rarely on plains.

Burrows as Habitat

Burrows serve a number of functions for animals such as crayfish. They provide: 1) shelter (from extremes in environmental conditions and from predators); 2) an

environment for interaction and reproduction; 3) water to moisten the gills for respiration and 4) a source of food (Growns 1986). Horwitz and Richardson (1986) classify Australian crayfish burrows into three types (Table 6.2.12 details these classifications, while Figure 6.2.2 illustrates them).

Many data categories from the Zoology collection referring to the burrows themselves could not be amalgamated to form larger groups on which analysis could be performed, and some data entries were ambiguous, for example "moderate" and "deep" could not be interpreted into actual depths and quantified. However, the data are sufficient to allow comment on trends. For example, from the collection data it appears that the majority of *Ombrastacoides* (Table 6.2.13) and all *Spinastacoides* (Table 6.2.14) species appear capable of constructing and utilising all but Type 3 burrows. Records for burrow type were insufficient for some of the *Ombrastacoides* species, so the absence of some species from the Type 1a and Type 1b categories may be an artefact.

Table 6.2.12. *A summary of the types of crayfish burrow (adapted from Horwitz and Richardson 1986).*

Burrow Type	Location in habitat	Derivation of water	Location
1a	in permanent water	permanent water body	Under rocks, ledges, in rock crevices in or under submerged logs and in short, unbranched burrows in the substratum of lakes, rivers and large creeks.
1b	connected to permanent waters	permanent water body	On the banks of permanent waters with openings above and below water.
2	connected to water table	groundwater + surface runoff	Constructed by digging down to the water table, with the burrows filling with interstitial water from the surrounding soil
3	independent of water-table	surface runoff only	Burrows never contact the water-table, with water derived from surface runoff.

Table 6.2.13. *A summary of burrow types from* Ombrastacoides *species from the Zoology collection data. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

Burrow Classification	Species
Type 1a	*dissitus, brevirostris, leptomerus, huonensis, decemdentatus*
Type 1b	*leptomerus, brevirostris, professorum, parvicaudatus*
Type 2	all species
Type 3	*

Table 6.2.14. *A summary of burrow types from* Spinastacoides *species from the Zoology collection data. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

Burrow Classification	Species
Type 1a	*insignis, catinipalma, inermis*
Type 1b	*insignis, catinipalma, inermis*
Type 2	*insignis, catinipalma, inermis*
Type 3	*

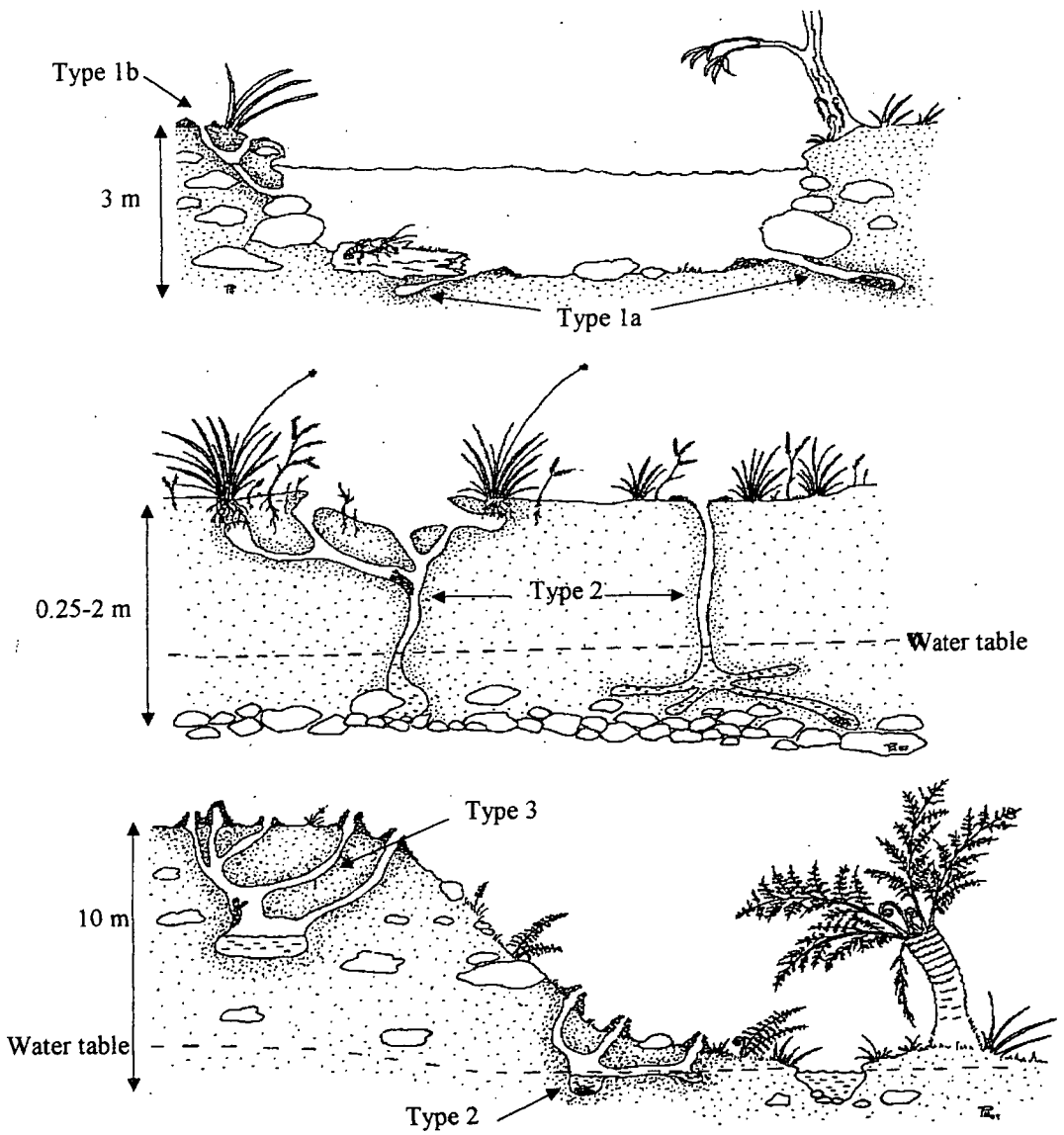


Figure 6.2.2. *Diagram of burrow systems (adapted from Horwitz and Richardson 1986).*

Data from the Zoology collection indicates a wide range of burrow depths (Table 6.2.15); burrows ranged in depth from mere runnels to 1.5 metres; descriptions citing "deep" burrows may imply depths of more than this. Richardson and Swain (1978) also mention depths up 1.5m in the Olga Valley, while Richardson and Wong (1995) note that burrows range in depth from a few centimetres in thin soils on slopes to more than two metres in poorly-drained flats. In areas where the water table is very low, for example in much of the distribution of *O. dissitus* and *O. denisoni*, burrows may approach two metres (pers. observ.).

Two species are described as having cryptic burrows (hidden, or difficult to find) in the records: *O. leptomerus* and *S. inermis*, however, personal experience suggests that this description could apply to more species, particularly in rainforest habitats.

Table 6.2.15. *A summary of burrow depths from both genera obtained from the Zoology collection data. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

Depth (records)	*Spinastacoides*	*Ombrastacoides*
0.3m	*	*brevirostris*
0.5m	*catinipalma, inermis*	*brevirostris*
0.6m	*insignis*	*
0.8m	*	*brevirostris*
1.0m	*insignis*	*
1.5m	*	*brevirostris*
runnel	*catinipalma*	*leptomerus, brevirostris, decemdentatus, huonensis, pulcher*
shallow, short	*catinipalma, inermis, insignis*	*leptomerus, asperrimanus, decemdentatus, parvicaudatus, professorum, brevirostris*
moderate	*catinipalma*	*
deep	*catinipalma, inermis, insignis*	*leptomerus, asperrimanus, ingressus, brevirostris, decemdentatus, huonensis, dissitus, denisoni*

The literature suggests that burrows often ramify extensively, with tunnels forming blind chambers; as these blind tunnels often terminate at the base of plants, it has been assumed that they are feeding chambers (Growns and Richardson 1988). Wong (1991) notes differences between burrows of *P.t. tasmanicus* (*O. huonensis*) at her Harlequin Hill study site and *P.t. insignis* (*S. insignis*) at her Melaleuca study site. In Wong's study the burrows of *O. huonensis* were generally quite extensive and included several entrances. The burrows start with a large surface "crater", several entrances leading to a terminal chamber; several blind, upward sloping chambers are

present near the surface. The burrows of *S. insignis* were usually more simple in construction, with fewer entrances. These burrows were never associated with craters, however they were often capped with a chimney.

Although limited, the Zoology collection data (Table 6.2 15) show *Spinastacoides* species occupying simple burrows, but no such records appear for *Ombrastacoides* species. However, there are records for *O. brevirostris* and *O. leptomerus* occurring "among pebbles in a roadside ditch", and several *Ombrastacoides* species occurring "under logs" or "under large stones"; we can assume that burrows associated with these descriptions could be classified as simple. The records note two *Spinastacoides* species, *S. catinipalma* and *S. insignis*, occupying "complex" or "ramifying" burrows, so these classification categories are not confined to *Ombrastacoides* species.

The records indicate at least two *Ombrastacoides* species having chimneys at the burrow entrance (Table 6.2.15), *O. brevirostris* and *O. asperrimanus*, with that of *O. brevirostris* being describes as very big. Several species are described as having ramifying (*S. catinipalmus, S. insignis, O. brevirostris*) or complex (*O. leptomerus, O. ingressus, O. huonensis* and *S. catinpalmus*) burrows. No *Spinastacoides* species are recorded occupying a burrow with an entrance crater, however, S. insignis may occasionally have a crater-like entrance (pers. Comm. Dr. A. M.M. Richardson, School of Zoology, University of Tasmania.

Table 6.2.15. *A summary of burrow complexities from both genera obtained from the Zoology collection data. The symbol * indicates the substrate type is not mentioned in the collection record and does not necessarily imply species cannot utilise this substrate.*

Complexity (records)	*Spinastacoides*	*Ombrastacoides*
simple	*insignis, inermis*	*
complex, ramifying	*catinipalma, insignis*	*brevirostris, leptomerus, ingressus, decemdentatus*
chimney	*	*brevirostris, asperimanus*
crater	*	*leptomerus*

As *Ombrastacoides* and *Spinastacoides* species often live within burrow systems that are not associated with free-flowing water, they are slow dispersers (see above).

Richardson and Swain (1991) found that over a 10 year study of a grid of 108 x 4 metre square quadrats containing 310 *Parastacoides* (now *Ombrastacoides huonensis* and *Spinastacoides inermis*) burrow systems, ~~no burrow systems either~~ appeared or disappeared. Burrow systems appear to survive longer than the life of a single occupant (Richardson and Swain 1991) and probably persist long enough to be occupied by a succession of individuals (Growns and Richardson 1988).

In summary, records from the School of Zoology collection giving details on the burrows of the two genera, whilst not as comprehensive as the vegetation records, nevertheless provide useful evidence of the extent of the burrow habitat of the species. Species from both genera appear to build Type 1a, Type 1b and Type 2 burrows; there is no record of any species constructing Type 3 burrows. Species from both genera occupy burrows of varying depth; from mere runnels to "deep". Species from both genera construct burrows that can vary from "simple" to "complex" or "ramifying", however, there is a tendency for burrows of *Spinastacoides* species to be simpler in construction. Species from both genera occasionally build chimneys on the burrow entrance, however only *Ombrastacoides* species excavate craters at the burrow entrance.

Diet

Studies on the diet of *Parastacoides tasmanicus* (*Ombrastacoides* and *Spinastacoides*) suggest that they are omnivorous, with a diet consisting mostly of decomposing vegetation, especially buttongrass fragments (*Gymnoschoenus sphaerocephalus*), roots, algae and a small amount of animal material (Lake and Newcombe 1975, Fradd 1979, Growns 1986, Growns and Richardson 1988).

In a study into the diet of *Parastacoides*, Lake and Newcombe (1975) mention that they used specimens of *P. tasmanicus* (most likely *O. huonesis*, but possibly including *O. pulcher*). Lake and Newcombe (1975) concluded that plant material formed the major part of the material found in the gastric mill, and that root material was the dominant part of the plant material. They also presented evidence of a cellulase enzyme in the crayfish gut, indicating that these crayfish are capable of obtaining soluble carbohydrate from plant material. The studies by Growns (1986) and Growns and Richardson (1988) were conducted at a site consisting of mostly

heathy sedgeland at Harlequin Hill, using what is now *O. huonensis*. Growns (1986) found a preference for decaying vegetation matter, and suggested that the presence of green and decomposing buttongrass fragments in the burrow may mean that the crayfish actively harvests material and waits for it to decompose. Growns and Richardson (1988) hypothesised that the low percentage of animal material in the diet of *O. huonensis* may be due to its low availability in the burrow system, rather than a preference for plant material.

Fradd's study (1979) was centred around the Scotts Peak Dam region, indicating that the specimens of "*Parastacoides tasmanicus*" used in this study were most likely *Ombrastacoides huonensis*; however, the study also included *S. insignis* and possibly *S. inermis* (pers. comm. Dr A. Richardson, School of Zoology, University of Tasmania), and these were not recognised during the course of the study. The vegetation at this site consists of mainly buttongrass and associated heath shrubs. In this comprehensive study into digestive enzymes and assimilation efficiency, Fradd (1979) found that the crayfish could digest animal material very efficiently (worms were assimilated with an efficiency of more than 85%, an efficiency equal to that of carnivores). The plant material used in his experiments, lettuce and boiled carrot, was assimilated with an efficiency of over 70%. This approaches the levels of assimilation efficiency shown by herbivores possessing enzymes capable of digesting the cellulose component of plants. Fradd found levels of both native and endogenous cellulase in the midgut and stomach of his specimens, but was unable to determine whether these were produced by the animals themselves, or by cellulase producing micro-organisms inhabiting the digestive tract. The specimens used in Fradd's study were capable of utilising all food types very efficiently, indeed, buttongrass mud was assimilated with an efficiency of more than 75%.

Detrital material, such as is found in buttongrass mud, can contain a high proportion of micro-organisms, as well as the decomposition products of plant and animal material, and micro-organisms may be the main food source for some detritivores. However, due to the slow rate of decomposition found in the anaerobic buttongrass peats, organic content matter is high and the micro-organism content would be expected to be low; Fradd concluded that micro-organisms may not form a substantial part of the diet of these crayfish. This does not, however, exclude the

possibility that the crayfish are deriving nutrients from bacteria or fungi associated with the rotting plant material they are ingesting.

Stable isotope analysis of specimens would enable the source of material assimilated into crayfish tissue to be determined. Studies of diet from different vegetation habitats are required to determine whether specialisation is occurring at the microhabitat level. This would be particularly useful in those species that have restricted ranges within the large *O. leptomerus* range: *O. parvicaudatus, O. ingressus* and *O. professorum.* Such a study would help to determine whether dietary specialisation is a determinant factor in the restricted range of these species. It is unfortunate that all the field studies pertaining to the diet of *Parastacoides* (now *Ombrastacoides* and *Spinastacoides*) species were conducted at sites dominated by buttongrass. *Ombrastacoides* and *Spinastacoides* species inhabit many other vegetation types, especially rainforest, and it is not known whether the way they utilise different vegetation types varies. Mature rainforest in Tasmania is a different trophic environment than buttongrass plains; typically little light penetrates the rainforest canopy, hence the ground cover consists mostly of mosses and ferns, with few leafy plants at ground level.

With the evidence of non-specialisation in vegetation habitat on the broad scale (see above), one can assume that *Ombrastacoides* and *Spinastacoides* species are able to obtain their nutritional requirements from any, or all, of these habitats and that vegetation type *per se* is not a limiting factor in the distribution of the two genera, or indeed a factor determining the distribution between species of the genera. It also appears that *O. huonensis*, at least, is able to obtain all their nutritional requirements from within the burrow system (Growns and Richardson 1988).

Ombrastacoides and *Spinastacoides* populations are sometimes found in sympatry with other freshwater crayfish genera, and it appears that when they are, they share a very similar diet. *Engaeus cisternarius* and *E. fossor* have ranges which overlap with that of *O. leptomerus*. A study by Suter and Richardson (1977) on the diet of these two *Engaeus* species found their diet to be virtually identical to that of *O. huonensis*: buttongrass, roots and dead wood dominated their diet, with occasional animal material. A study into possible habitat partitioning between *Ombrastacoides*,

Spinastacoides and *Engaeus* species occurring in sympatry may shed some light on whether these crayfish compete for a common food source.

Temperature

Records from the Zoology collection include specimens of *Ombrastacoides* and *Spinastacoides* collected from sea-level to alpine areas above 1000m. It appears that some *Ombrastacoides* species are able to tolerate colder conditions than, for example, *Engaeus* species; near areas where *Ombrastacoides* and *Engaeus* species are in sympatry *Ombrastacoides* species are able to extend their range higher into the mountains than *Engaeus* species (pers. comm. Dr A. Richardson, School of Zoology, University of Tasmania, pers. observ).

According to Langford (1965), at an altitude of 450m in inland regions of Tasmania, the average maximum day-time temperature falls below 10°C for two months of the year; at 1000m this occurs 6.5 months of the year. Above 300m there is no frost-free month. Coastal regions are milder, with days having a maximum temperature below 10°C rare. Figure 6.2.3a and 6.2.3b show mean summer maximum and minimum winter temperatures in Tasmania. Comparison of these maps and the distribution maps (Figures 6.1.5 and 6.1.6) shows that *Ombrastacoides* and *Spinastacoides* species can persist within wide temperature fluctuations: from average summer maxima of 18°C to average winter minima below 0°C. Extreme temperatures outside this range can occur, with the extreme maximum of approximately 40°C and the extreme minimum of approximately -10°C recorded in some areas of *Ombrastacoides* and *Spinastacoides* distribution (Langford 1965).

In his study on aspects of the ecophysiology of *Parastacoides tasmanicus*, Fradd (1979) found that these crayfish were adapted to a wide temperature range, and while lower temperature (5°C) appeared to affect enzyme activity etc, the animals regulated their activities well accordingly. The present-day distributions of most species of the two genera encompass a wide range of temperatures, with all but a few species extending their range into the subalpine regions. The exceptions are the some species with restricted ranges: *O. ingressus*, *O. dissitus* and *O. denisoni*. The large temperature range tolerated by the wide spread species suggest that, while most

populations are found in relatively temperate conditions, most species of these are capable of tolerating sub-alpine conditions.

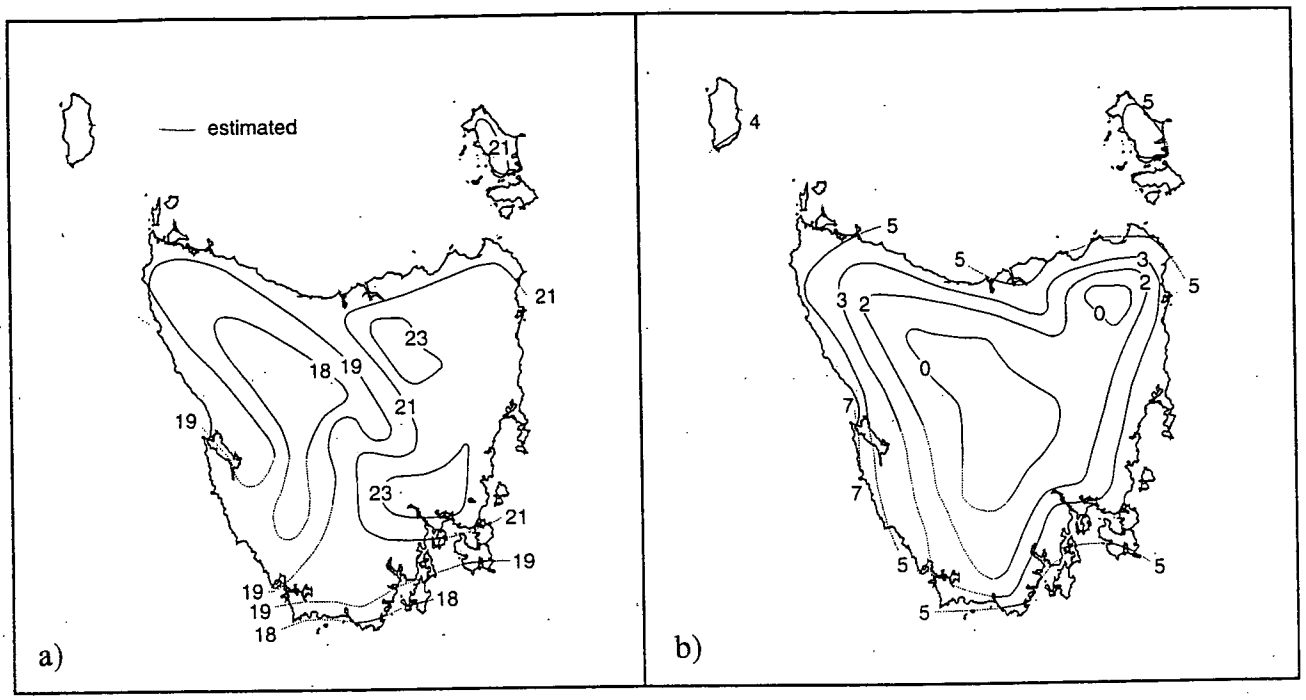


Figure 6.2.3a. *Shows the mean maximum temperatures in January (summer).*

Figure 6.2.3b. *Shows the mean annual temperature in July (winter). Temperatures are in degrees Celsius (from Jackson 1999b).*

Water availability

In a study into the respiratory responses to hypoxia in two Tasmanian crayfish genera, Swain *et al.* (1987) found that a *Parastacoides tasmanicus tasmanicus* species (*O. huonensis*, possibly *O. pulcher*) was able to survive for several weeks in burrows without water. Further studies (Fradd 1979, Swain *et al.* 1988) suggest that while *Ombrastacoides* species can survive periods without free-standing water they are nevertheless dependent on water for the majority of their respiratory requirements, as they have no apparent morphological adaptations for respiring in air. Fradd (1979) found that even when the burrow has no free water, the relative humidity within the burrow rarely drops below 100%.

The gill structure of *O. huonensis* appears to be modified, allowing the crayfish to use not only very turbid water, but also the hypoxic water often found in burrows

(Swain *et al.* 1988). This *Ombrastacoides* species was also able to regulate the amount of oxygen consumed until the oxygen level in the water drops to about 4 mL/L. Below this level the crayfish appear to reduce their activity to suit the level of oxygen available (Fradd 1979). When the level of oxygen drops below levels where these adaptations are useful, approximately 0.8 mL O_2/L at 15°C, the crayfish leave the water and spend a large proportion of time respiring in humid air. The crayfish can survive 15-20 hours at 15°C, and up to 60 hours at 5°C, in anoxic conditions, then the lactic acid produced during this time is rapidly excreted once conditions improve.

Over much of western Tasmania, annual rainfall exceeds the rate of evaporation, however there are some areas where this may not be the case for one to two months during the summer (Jarman *et al.* 1988). A distinct rain shadow effect is evident in central, eastern and southeastern Tasmania due mainly to the effect of mountain ranges, which disturb the generally westerly rain-bearing regime (Langford 1965). Western Tasmania receives abundant rainfall every month of the year, whereas eastern Tasmania has a lower overall rainfall, with distinct relatively dry months; for much of the eastern part of the state, evaporation is close to, or exceeds the annual rainfall (Langford 1965). Figure 6.2.4a and 6.2.4b indicate the mean annual rainfall and the mean annual evaporation rate in Tasmania.

The eastern boundary of the distribution of *Ombrastacoides* and *Spinastacoides* (Figure 6.1.4) closely approximates the 1000mm isohyet (Figure 6.2.4a). The eastern boundary of the distribution of *Ombrastacoides* and *Spinastacoides* also closely approximates the region in the east-west climatic gradient at which evaporation exceeds rainfall (Figure 6.2.4b). The present distribution of the species west of the 1000mm isohyet and the necessity for burrows to contain water throughout most, if not all, summer months, suggests that lack of soil moisture, particularly in the summer months, may limit the possibility of expansion much further eastward. Whilst these crayfish may be able to survive short periods without water, long-term or repeated dry spells are probably fatal. The 1000mm isohyet (or Tyler's Line) is a rough approximation of a barrier for the eastward dispersal of species of these two genera.

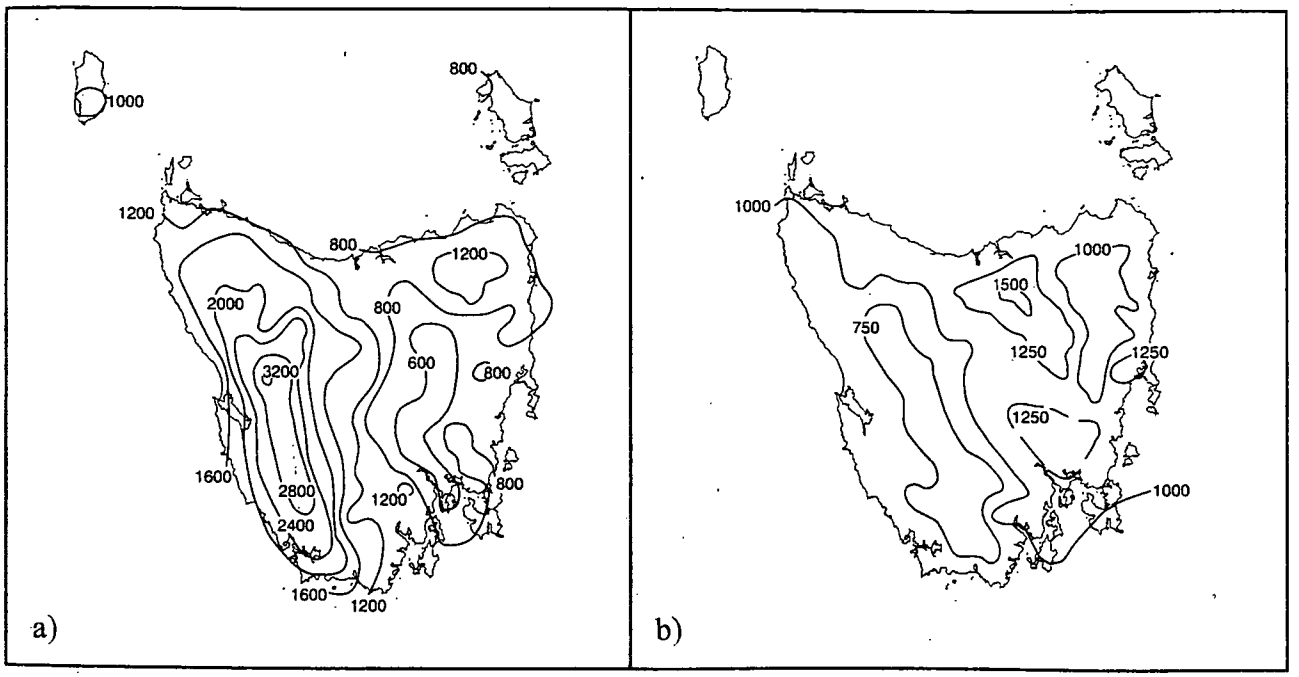


Figure 6.2.4a. *Shows the mean annual rainfall in millimetres.*

Figure 6.2.4b. *Shows the mean annual evaporation rate in millimetres (from Jackson 1999b).*

Historical factors influencing present-day distributions

The world's climate is not stable; it has varied dramatically over time. Milankovitch oscillations, which occur every 10-100 thousand years, produce large and rapid temperature and precipitation changes (Dynesius and Jansson 2000). These changes increase in intensity towards the poles, and have influenced the distribution of both flora and fauna. Studies in the Northern Hemisphere suggest that Pleistocene glacial events were responsible for the origin of many present-day species and/or their present distributions, in a number of taxa ranging from birds (Blondel *et al.* 1996, Merila *et al.* 1997, Wenink *et al.* 1996) and insects (Bilton 1994, Brower 1994) to fish (Avise 1992, Bernatchez and Dodson 1991). However, Klicka (1997) suggests that many North American songbirds previously thought to be of Pleistocene origin show instead a protracted history of speciation events over the past five million years.

Although glacial events were not as severe in the Southern Hemisphere, Pleistocene glacial events have been suggested to be responsible for the speciation and distribution of some *Engaeus* species of freshwater crayfish (Horwitz 1988). However, the molecular studies presented here (see Chapters 2 and 3) suggest that speciation events for the crayfish species which are the subject of this thesis are far more ancient.

While Pleistocene glacial events can be eliminated as agents promoting speciation, species of the two genera *Ombrastacoides* and *Spinastacoides* are now distributed in areas which have been affected by Pleistocene (and earlier) glacial events, and so is relevant to ask whether their present-day distributions reflect these events. Macphail *et al.* (1993a) suggest that the climatic changes wrought by the Pleistocene created environmental stresses such as cold, periglacial activity and aridity not previously experienced during the Cenozoic. The ways in which glaciation might have caused habitat disruption for *Ombrastacoides* and *Spinastacoides* include many environmental and physical stresses: vegetation change, temperature decline, aridity, periglacial activity, steepening of mountain and valley slopes, and slope instability. These physical impacts are noted by Kiernan (1990b) around the Mt Anne Massif and Schnells Ridge, a region now inhabited by *Ombrastacoides* species.

There have been at least four glacial events associated with the Pleistocene in Tasmania (Kiernan 1990a), with evidence in some areas, such as the upper Franklin Valley, for as many as six (Kiernan 1989). Whilst the rugged terrain of Tasmania makes detailed study of glacial evidence difficult, recent studies have resolved many questions regarding the history of glacial events in Tasmania (Kiernan 1990a). Table 6.2.16 details known glacial events in Tasmania, while Figure 6.2.5 summarises these events in diagrammatic form, indicating the timing of the glacial and interglacial events. Figure 6.2.6 indicates areas in Tasmania known to have been glaciated. The most severe glaciations appear to have occurred in the Early and Middle Pleistocene or Pliocene (Kiernan 1990a). During the Pleistocene, there have been times when ice up to 600m thick in places stretched continuously over the Central Plateau to the West Coast Range (see Figure 6.2.7). It has been estimated that this ice cap, and the valley glaciers associated with it, covered an area of approximately 6000 square kilometres (Kiernan 1990a).

The westerly precipitation-bearing air stream present today was also the major influence during the Pleistocene; the heaviest snow build up was in the Pieman headwaters, rather than further east on the Central Plateau (Kiernan 1990a). Colhoun *et al.* (1996) quotes Davis as suggesting that the snowline was lowered by approximately 1000m during the Late Pleistocene glaciation, while the temperature was lowered by 6 – 6.5°C. The Late Pleistocene glaciation began approximately 26-25 ka BP. The maximum extent of the ice was reached approximately 19 ka BP. All but the highest cirque glaciers had vanished by 10 ka BP (Colhoun *et al.* 1996).

Different areas of Tasmania appear to have been affected to differing extents during glacial events; ice limits in the Pieman headwaters were closer to the maximum extent in the Middle Pleistocene than they were in the southern or northern regions at the same time (Kiernan 1990a). The climate between glacial events was not constant, and there is evidence to suggest that the period before the last glacial event was as much as 5°C colder than the present (Jackson 1999c); this would imply a tree line near the present sea level.

The moraines at Lake St Clair suggest that deglaciation in that area occurred in stages, whereas in the West Coast Range deglaciation appears to have occurred in one rapid event (Kiernan 1992). This suggests that the rate at which new habitat became available to *Ombrastacoides* and *Spinastacoides* species through deglaciation was not the same throughout Tasmania, and estimates of range dispersal would vary accordingly.

Evidence that broad climatic changes have occurred in Australia, and in particular in Tasmania, during the Pleistocene is apparent when examining the fossil flora. Rainforest provides an important habitat for both *Ombrastacoides* and *Spinastacoides* species (see above), and this habitat has been greatly impacted by Pleistocene glacial events in regions now within the range of these genera. There is evidence of local extinctions; Early Pleistocene rainforest appears to have had a higher level of species richness than Middle and Late Pleistocene rainforest (Hill and Jordan, 1997). Fossil evidence from Regatta Point (see Fig. 6.2.9) suggests that while

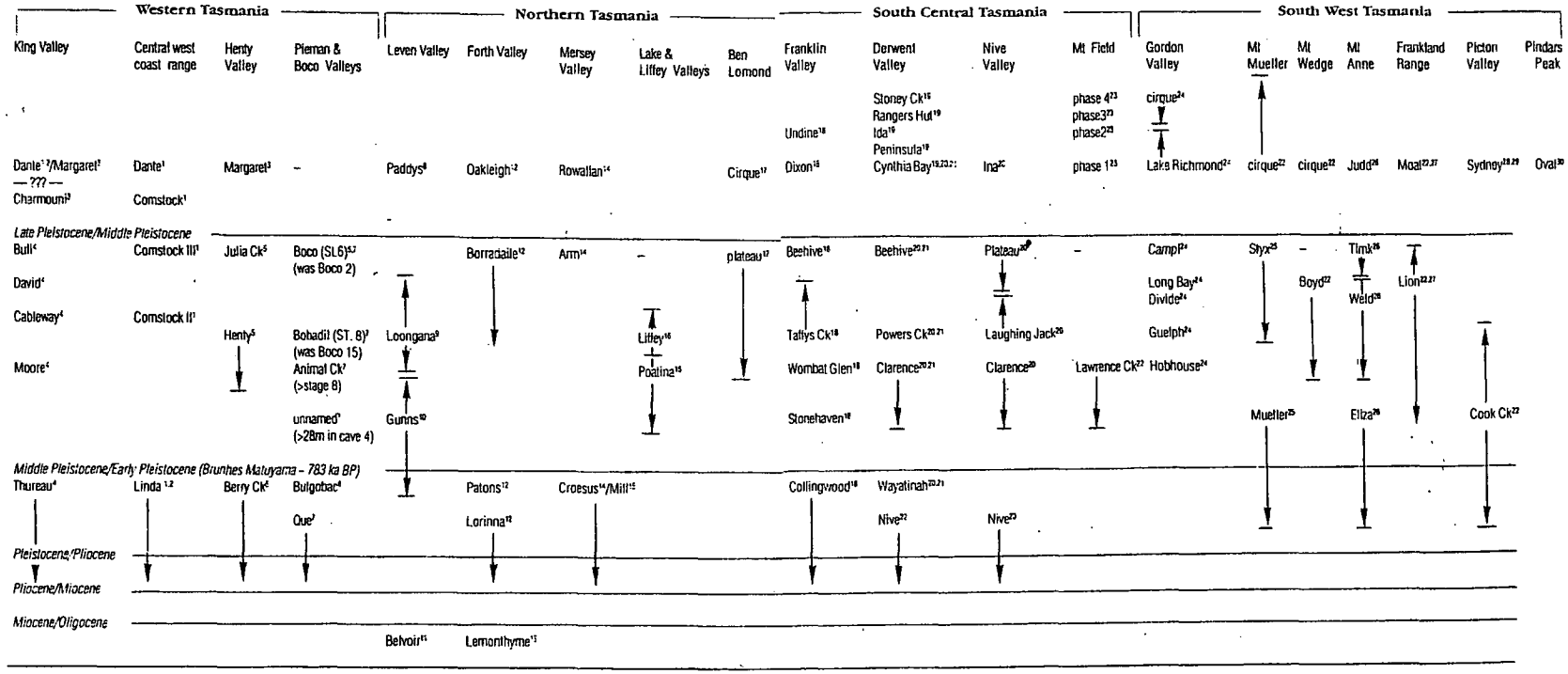


Table 3.3 Nomenclature of glacial advances recognised in Tasmania to 1995 and suggested broad age relationships (precise correlation is not implied).

Dashed vertical lines indicate most likely alternative time range.

Sources:

1 Kiernan 1980
2 Kiernan 1983a, b
3 Lewis 1945, Colhoun 1985
4 Fitzsimmons 1988, Fitzsimmons *et al.* 1993
5 Augustinus and Colhoun 1985
6 Augustinus 1982
7 Augustinus *et al.* 1994
8 Kiernan 1990a
9 Kiernan and Eberhard, in press
10 Kiernan, unpublished
11 Augustinus and Idnurm 1993
12 Kiernan and Hannan 1991
13 Macphail *et al.* 1993
14 Hannan 1989, Hannan and Colhoun 1987
15 Kiernan 1984
16 Hannan 19..
17 Caine 1983
18 Kiernan 1989b
19 Kiernan 1992b
20 Kiernan 1985
21 Kiernan 1991b
22 Kiernan, unpublished
23 Macintosh 1993
24 Kiernan 1995b
25 Kiernan 1993a
26 Kiernan 1990c
27 Kiernan 1994a
28 Colhoun and Goede 1979
29 Kiernan 1989e
30 Kiernan 1987b

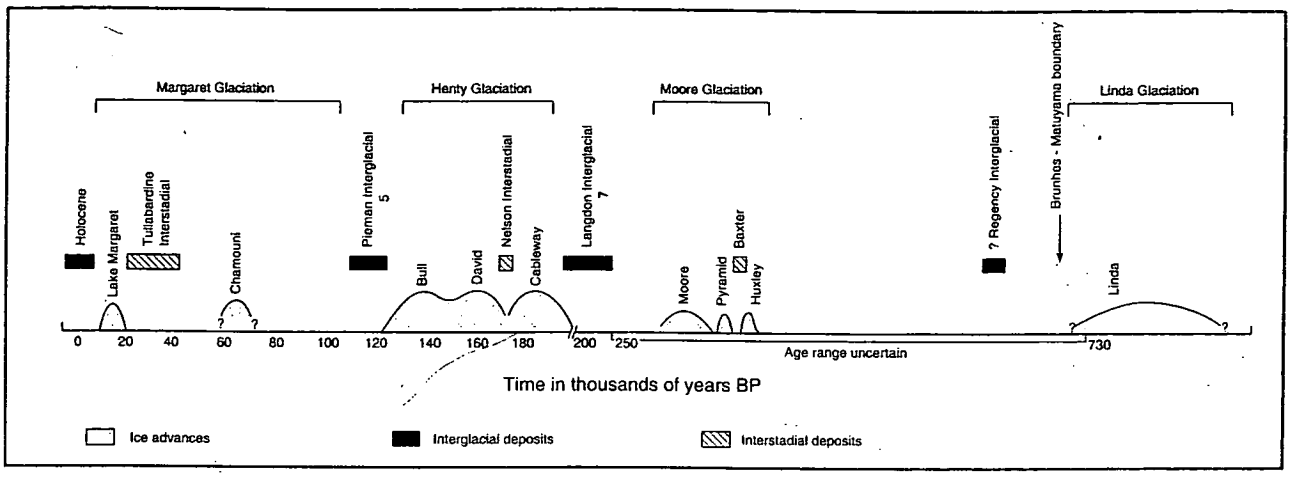


Figure 6.2.5. Model of glacial events in Tasmania (from Colhoun and Fitzsimons 1990).

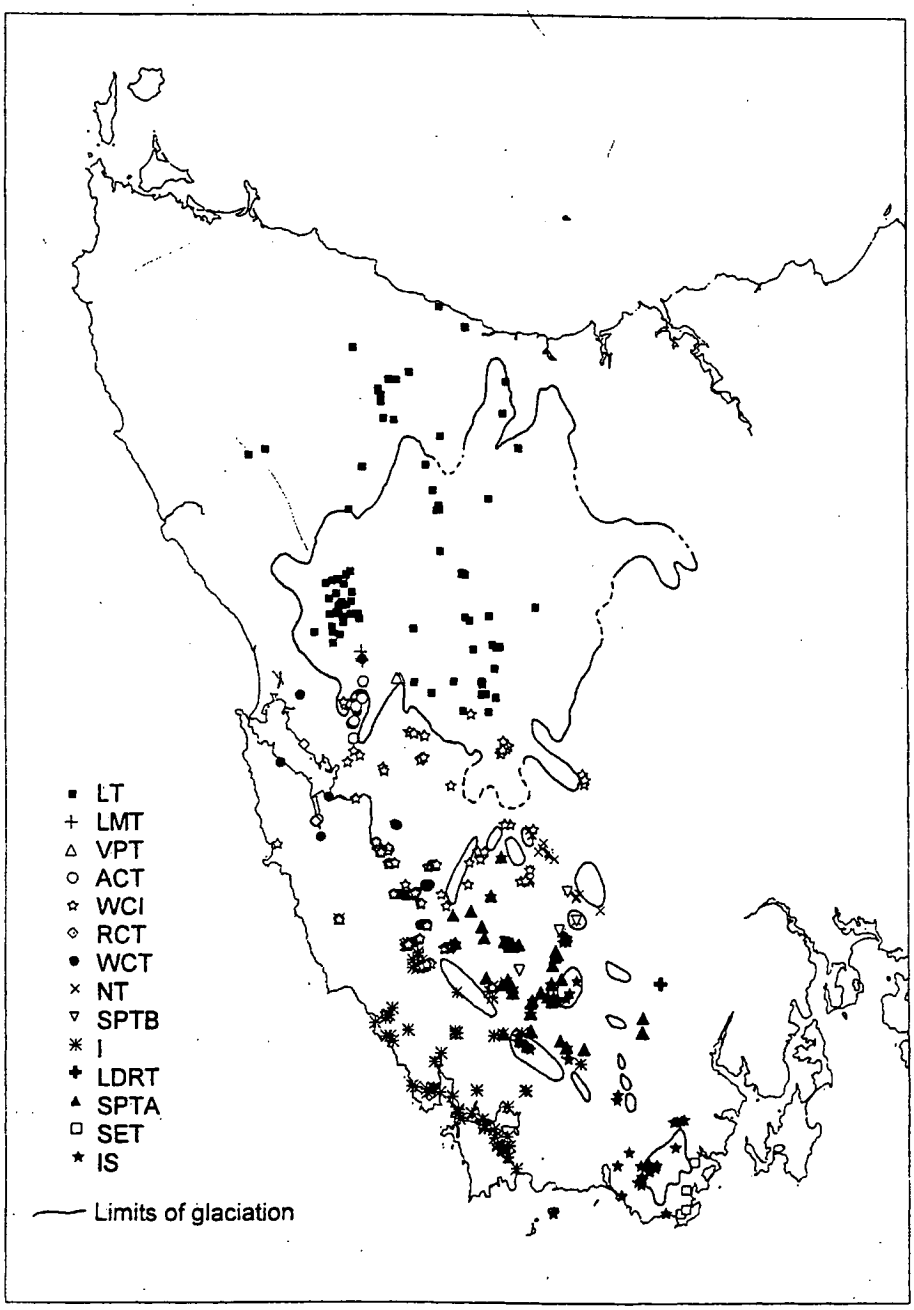


Figure 6.2.6 Map of known glacial maximums in Tasmania (from Hansen and Richardson 1999).

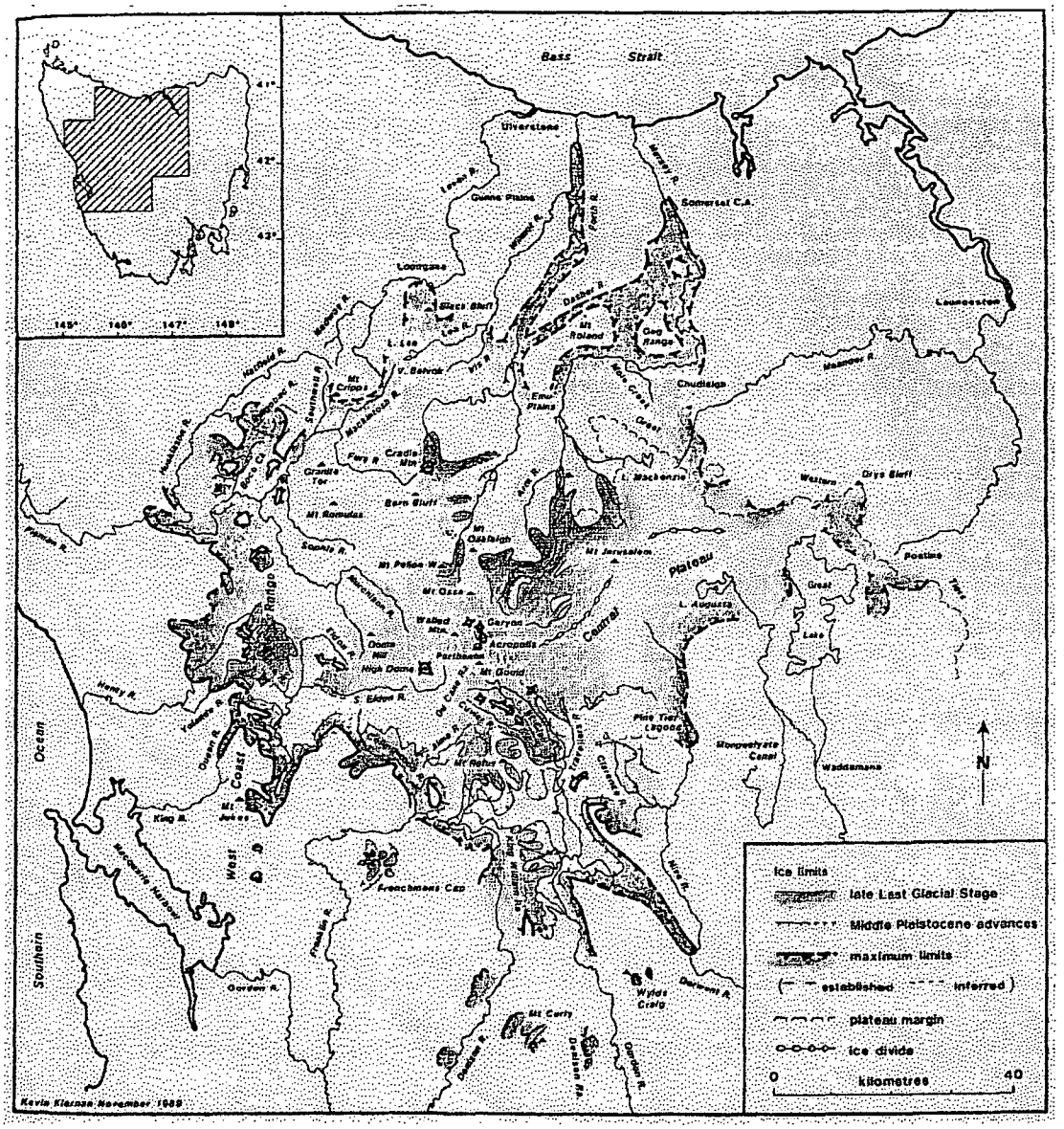


Figure 6.2.7. *Ice limits in the Tasmanian Central Highlands (from Kiernan 1990a).*

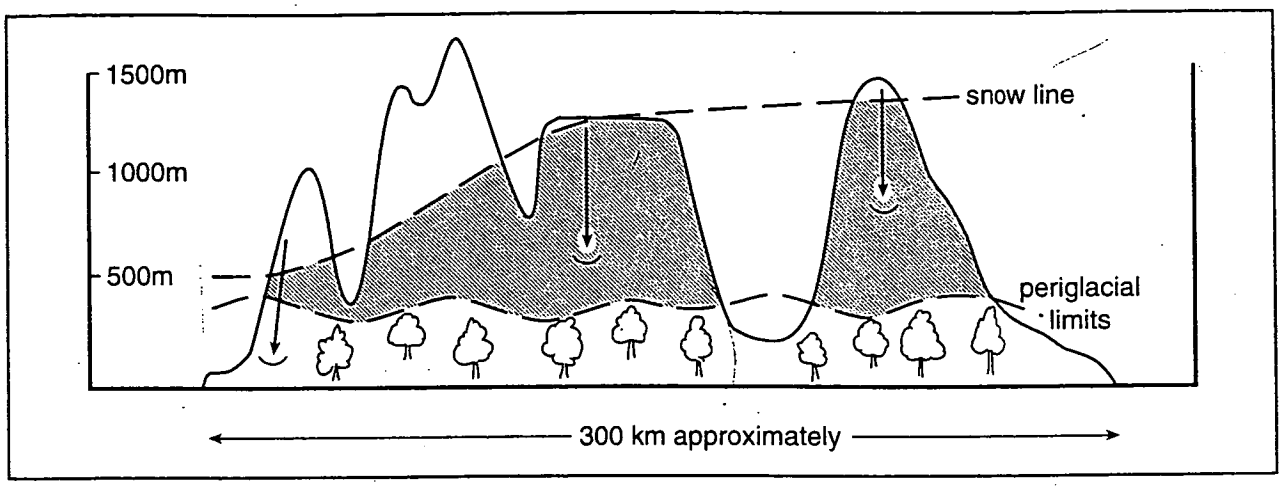


Figure 6.2.8. *Approximation of the snowline along the length of Tasmania during the last glacial event (from Jackson 1999c).*

the region may have been cold and dry during Early cold phases, rainforest trees (Jordan *et al.* 1995) and mosses (Jordan and Dalton 1995) were present, possibly in wet, riparian regions. All the major river systems in western Tasmania between the Huon and the Pieman, as well as some of the deep inland western valleys, would have been refugia for rainforest vegetation during the Pleistocene glaciations (Kirkpatrick and Fowler, 1998). These riparian shelters may have provided refugia for *Ombrastacoides* and *Spinastacoides* species during the coldest phases of glacial events. While there is evidence of plant species extinction, there is also evidence of plant communities habitat-tracking as conditions changed. Evidence from Regatta Point also suggests that at times during cold phases the plant community resembled that of modern undisturbed subalpine western Tasmania (Hill *et al.* 1999); these communities were at sea level during the Early-Middle Pleistocene (see Fig 6.2.10). Some *Ombrastacoides* and *Spinastacoides* survive in subalpine conditions today, so it is reasonable to assume that they did so during glacial events as well.

Conditions necessary for fossil formation favour rainforest species and there is only limited information available for other vegetation types (Hill *et al.* 1999). There is little fossil evidence of the distribution of buttongrass communities during the Pleistocene period, however, one can assume that conditions necessary for these communities today would, to a large extent, also be necessary during the Pleistocene. This argues for a greatly restricted distribution of buttongrass communities for most of the Pleistocene.

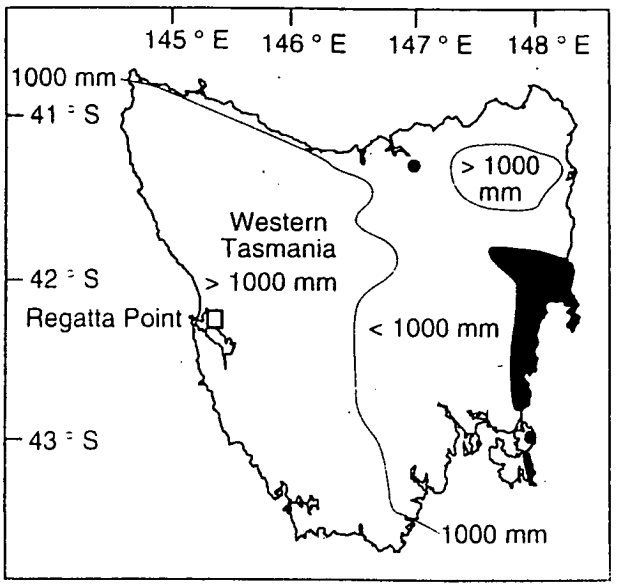


Figure 6.2.9. *Map of Tasmania showing Regatta Point fossil bed location (from Jordan 1995).*

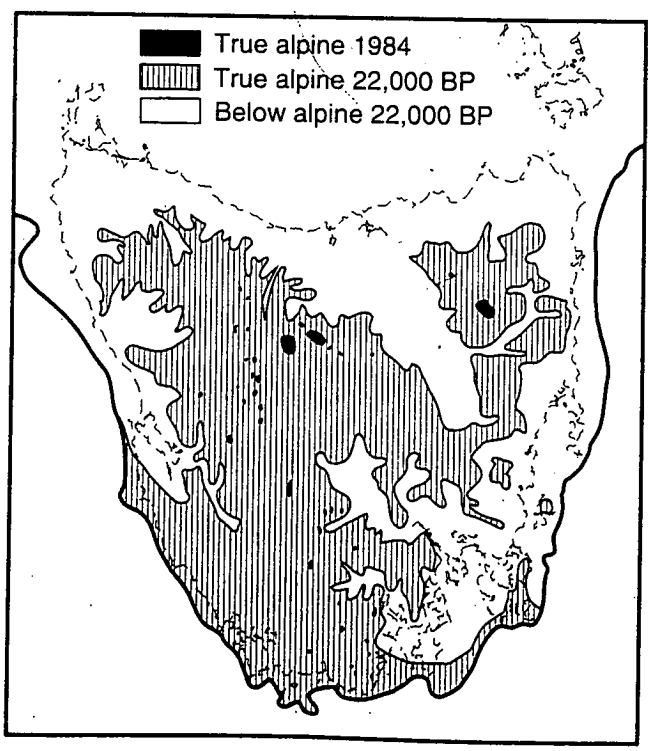


Figure 6.2.10. *Environments based on a drop in the mean temperature of 5.5℃ (10℃ equals tree line) (from Jackson 1999c).*

Pleistocene glaciations, in particular the early glaciations, affected the distribution of *Ombrastacoides* and *Spinastacoides* species by restricting their range, and perhaps creating local extinctions, in parallel with one of the main habitats of these crayfish,

rainforest. However, later glacial events were not as severe and crayfish distributions may not have been much reduced from present-day ranges in much of the state.

Discussion

Data from the Zoology crustacean collection provide no evidence of ecological factors that may be influencing the distribution of the individual species. Species of both genera are able to occupy burrows in a range of conditions, however there is a tendency for *Spinastacoides* species to occupy drier slopes. The evidence indicates that the species with large distributions from both genera are capable of inhabiting all the vegetation types they are likely to encounter; there do not appear to be any vegetation habitat specialists. For the species with restricted distributions, there is no evidence to suggest mechanisms restricting their distribution. However, I suggest that competitive exclusion may prevent some species from utilising vegetation where species occur in sympatry, either in SCZs or at the borders of exclusive restricted distributions.

Most species of both genera appear to be dietary generalists; they are able to find a suitable food source in most environments found in southern and western Tasmania. Studies on the feeding ecology of *Ombrastacoides* have only been carried out in buttongrass habitats, but as they are found in great numbers in other habitats, notably heathlands and rainforests (Hansen and Richardson 1999), we can assume that food resources are adequate in these vegetation types.

While glacial events which occurred during the Pleistocene (and Late Pliocene) have produced environmental changes causing dramatic shifts and extinctions in the flora of western Tasmania, they appear to have had little impact on *Ombrastacoides* or *Spinastacoides* species. Dynseius and Jansson (2000) note that in regions of steep altitudinal gradients, as is the case for much of western Tasmania, species often do not need to move long distances to experience marked environmental shifts. They suggest that regions experiencing large changes in climate during these oscillations should have organisms that are generalists, that is, they do not need to disperse as fast or as far as organisms tracking specialised habitats. The present-day ranges of many *Ombrastacoides* and *Spinastacoides* species extend from coastal to sub-alpine regions, and from dry heathlands and waterlogged buttongrass plains to rainforest, suggesting that these species are habitat generalists. Glacial refugia, for these species

at least, need only to have been as warm as present alpine conditions for some populations of these species to survive. Conversely, when more hospitable habitats were in shorter supply, populations of cold-tolerant species may have been more wide-spread in subalpine areas than is the case at present. These populations of cold-tolerant species may not have needed to habitat-track during glacial range restrictions of heathland or rainforest flora to gain refugia, they may simply have stayed in place utilising subalpine flora rather than heathland or rainforest flora.

In summary, whilst the interaction of rainfall and evaporation rate appears to determine the eastern boundary of the distribution of *Ombrastacoides* and *Spinastacoides* as a whole, soil moisture content, temperature and vegetation do little to explain the distribution of the individual species, except where they occur in sympatry. Pleistocene glacial events appear to have had minimal influence in restricting the range of suitable habitats for these species, except in the very early Pleistocene. The ranges of individual species may still be expanding, and none of the parapatric boundaries appear to be associated with physical or environmental barriers to further expansion. I suggest the underlying pattern in the distribution of the different species must be related to events either before or during the Early Pleistocene glaciations, and most likely reflects ancient speciation events during the Miocene period. While sympatric contact zones may represent recent interactions between species (for example *S. catinipalma* and *S. insignis*), the habitat-partitioning seen between species in these zones argue for long established relationships.

7. General Discussion

Taxonomic review

This study has shown that the taxonomy of the genus *Parastacoides* is more complex than previously thought. The genus has had to be considerably revised; two new genera comprising fourteen species are now recognised (see Chapter 5 for descriptions and keys). The revised taxonomy is supported by molecular (Chapters 2 and 3) and morphological (Chapters 4 and 5) studies. The main diagnostic feature separating the two genera is the presence/absence of a terminal median spine on the uropod endopod exopod; a terminal spine is present on species in the new genus *Spinastacoides*, and species without the terminal spine are now placed in the new genus *Ombrastacoides*. The new classification resolves the nomenclature complication arising from the incorrectly attributed genotype (see Chapter 5).

These studies partially validate earlier work by E.F. Riek; through a series of reviews Riek revised the number of species from three (Clark 1941) to five, seven and finally six (Riek 1951, 1967, 1969). I agree with his designation of *P. leptomerus* and *P. pulcher* as separate species, however, I find that *P. setosimerus* is synonymous with *P. leptomerus* (Riek synonymised *P. setosimerus* with *P. tasmanicus*) and that *P. sternalis* is synonymous with *P. inermis*. Nevertheless, the suggestion that *Parastacoides* was more speciose than the current taxonomic status suggests (one species consisting of three subspecies (Sumner 1978)), was correct.

Sumner (1971) used numerical taxonomy (Weighted Variable Group Method) to evaluate the extent of similarity between his taxonomic units. He pointed out that the many phenotypically overlapping characters present in the genus made some common numerical methods difficult to compute. I suggest that these methods, while useful when dealing with taxa which exhibit a large number of taxonomically distinct characters, are not useful when dealing with taxa which are as morphologically conservative as freshwater crayfish, and a combined molecular and morphological approach is necessary to resolve the taxonomy fully. Several recent reviews of freshwater crayfish (Austin 1986, Horwitz 1990, Ziedler and Adams 1990, Horwitz

and Adams 2000) have used the approach of combining molecular and morphological analyses in determining taxonomic status. This approach is particularly appropriate when dealing with morphologically conservative taxa.

It is worth remarking that Sumner (1978) found that up to 90% of individuals in some populations (which he did not identify) were intersexed (having sexual characteristics of both sexes). However, intersexed individuals did not occur in high numbers in any populations in my study (see Chapter 5). A study into the true extent of intersexed individuals may prove worthwhile to determine whether high numbers are a local phenomenon, or whether environmental factors influence the proportion of intersexed individuals occurring in the population, and also to determine the functional sexuality of intersex individuals. Horwitz (1990) found some species of *Engaeus* to be almost always intersexed, and used this feature as a diagnostic character, so we may assume some genetic control of intersexuality in those species, however, little work has been done on intersexuality in parastacids. Intersexuality in crustaceans can take several forms: protandry (change of sex from male to female), protogyny (change of sex from female to male), or bilateral separation (Sagi *et al.* 1996). In several species of Australian parastacids individuals possess both male and female openings (Sagi *et al.* 1996). Sagi *et al.* (1996) examined intersexed specimens of *Cherax quadricarinatus* and found that these specimens were functionally male; true hermaphroditism was not observed. In this species, the intersex phenomenon appeared to be stable, as specimens kept in the laboratory for 19 months did not change. In a later experiment, Sagi *et al.* (1999) reported that removal of the androgenic gland from intersex specimens significantly increased the production of vitellogenic-specific protein, permitting secondary vitellogenesis, suggesting that sexuality may be plastic in some species.

Speciation and Evolutionary History

Early hypotheses regarding the evolutionary history of the two genera suggested that Pleistocene glacial events had significant impacts on both speciation (Sumner 1978, Hansen and Richardson 1999a) and distribution (Knott 1975, Hansen and Richardson 1999b). The lack of fossil material is a hindrance to the elucidation of the evolutionary history of these genera. Assuming that these genera have always

inhabited similar environments as they do now, it is unlikely that fossil evidence will be found, as they usually inhabit very acidic water (pH > 3.7) (Newcombe 1975).

I suggest that the phylogeny based on the morphological characters is the least robust of the phylogenies developed. This is because the taxa are morphologically conservative, yet exhibit a large degree of within species variability, and so it is not possible to make judgements concerning homoplasy, i.e. which similarities are due to convergent evolution and which similarities are due to having a common ancestor.

There are at least two competing theories on modes of speciation: geographical (Bush 1975) and evolutionary-mechanistic (Templeton 1981). While there is some overlap between them, they cannot be equated (Templeton 1998). Bush (1975) states that speciation is ultimately an adaptive process involving the establishment of inherent barriers to gene flow by the development of reproductive isolating mechanisms. Bush (1975) suggests four models of geographical speciation.

1) Speciation by subdivision (also known as vicariant or allopatric Type 1), where a widespread species is fragmented, giving rise to daughter species each having a large range. This method usually requires an extended period of isolation, during which many small genetic changes accumulate, however chromosomal rearrangement may lead to rapid allopatric speciation.
2) Speciation by founder effect (peripatric or allopatric Type 2), where peripherally isolated daughter species have a limited range. These species often result from a population flush during a period of rapid population increase; speciation may occur rapidly and there may be little ecological change.
3) Speciation in a cline (parapatric); no spatial isolation is necessary, the level of vagility is usually very low and reproductive isolation arises with the exploitation of new habitats by genetically unique individuals.
4) Speciation by moving to a new niche, causing pre-mating reproductive isolation (sympatric). This may be the result of a change in pheromones, timing of mating or host (habitat) shifts (eg *Rhagoletis* (Smith and Bush 1998)).

Templeton (1981) suggests two modes of evolutionary-mechanistic speciation: divergence and transilience.

Divergence speciation can be divided into three modes:

1) adaptive, where a population is divided by an extrinsic barrier to gene flow, which does not need to be geographical,
2) clinal, where isolation of geographically separated sections of continuous populations occurs, and
3) habitat divergence, where parts of a single breeding population become adapted to a new habitat.

Transilience speciation occurs when there is

1) chromosomal transilience, where a chromosomal change occurs,
2) genetic transilience, where a founder event produces rapid changes in a previously stable genetic system,
3) hybrid maintenance, or
4) hybrid recombination.

Much of the following discussion is based on the geographical theory of speciation; the genetic analyses required to support Templeton's theories were not attempted in this study. Based on Bush's (1975) theory of speciation, a number of hypotheses can be raised to explain the evolution of *Ombrastacoides* and *Spinastacoides* based on their geographical distributions and the phylogenies erected by the various methods used in this thesis.

The first hypothesis involves vicariance (allopatric Type 1 or adaptive divergence) – after the crayfish ancestral to both *Ombrastacoides* and *Spinastacoides* had evolved, it dispersed throughout Tasmania. Environmental conditions changed (see below) and populations became isolated; this vicariant event resulted in allopatric speciation in the isolated remnant populations. Templeton (1981) suggests that even when ancestral populations occupy a similar habitat, fragmentation of the populations may lead to incipient speciation. The regular climate oscillations during the Pleistocene then maintained the distribution of the species; the relatively short periods between glacial events not allowing expansion beyond current distributions. This waxing and waning of the species may have resulted in short periods of contact, but little mixing. If this hypothesis is correct, one would expect closely related sister taxa to be close geographically, and this does appear to be the case for most groups. Also, if there

were only one major vicariant event responsible, for example, the increasing aridity seen during the Miocene, one would expect branch lengths leading to the terminal points to be more or less the same length, and there should be few internodes (Ponniah and Hughes 1998). However, this is not the case; branch lengths vary, suggesting more than one speciation event.

Another hypothesis is that of a step-wise expansion of the range by successive species (allopatric Type 2). This may be the result of a population 'flush' producing semi-isolated peripheral populations (Bush 1975). These peripheral populations are more likely to be close to unexploited habitats suitable for invasion; speciation may then occur rapidly with little ecological divergence. This would also imply that closely related sister taxa should be close geographically, however there should be an obvious basal species, and the phylogenetic tree would not be symmetrical (Ponniah and Hughes 1998). The geographic location of the basal species would also indicate to some extent the origin of the group, and the most recently evolved species should be the most geographically distant from the basal species. This scenario does not fit the phylogenetic trees produced in the course of this study, suggesting that the evolutionary history of these genera is more complex than this hypothesis allows.

A further hypothesis would be that of speciation by adaptive radiation (a parapatric model). Populations, particularly those at the edges of the range, may be exposed to novel habitats as their range expands; they adapt to the new environments and become isolated from the rest of the population. However, this study suggests that the diversity of *Ombrastacoides* and *Spinastacoides* cannot be explained by adaptive radiation (Hansen and Richardson 1999a), since all species appear to be generalists in their habitat requirements. The only factor limiting the distribution of the group appears to be the combination of rainfall and evaporation rate (Chapters 6.1 and 6.2). So long as sufficient moisture levels are retained in the burrow systems for sufficient periods over the drier summer months, the crayfish appear able to adapt to virtually all vegetation, soil, altitude and temperature conditions available to them. Therefore, a hypothesis based on adaptive radiation seems unlikely.

A final hypothesis might involve a combination of the first two hypotheses; one or more vicariant events isolating populations, and the new species experiencing range

expansions and isolation. If this were the situation, sister taxa would not necessarily be closely related geographically, as range disruptions during vicariant events may produce extremely isolated pockets, and further range expansion may not reach these populations for some time. There would still be some structure present in the phylogenetic tree to hint at periods of isolation and some sister taxa may still be close geographically (Ponniah and Hughes 1998). The explanation that best fits the phylogenetic trees and the geographical distribution of the species is that of a multiple vicariance and range expansion scenario. This is based on an interpretation of a combination of the two allozyme phylogenetic trees and the 16S tree. A hypothesis to explain the possible evolutionary history of *Ombrastacoides* and *Spinastacoides* can be developed based on this evidence.

The ancestral species to both *Ombrastacoides* and *Spinastacoides* evolved some time before the Miocene (~23-5 Mya). Lack of fossil evidence precludes any assessment of, for example the mandibular structure, that might indicate whether these taxa may have been generalists in their habitat requirements. Rainforest (a habitat suitable for *Ombrastacoides* and *Spinastacoides* species) was widespread in Tasmania during the Eocene (~35-56Mya) (Hill *et al.* 1993), and during the Oligocene-Early Miocene (~30-20 Mya), conditions on the Central Plateau (around 700 metres above the sea-level at that time) were cool, moist and well below the tree-line, supporting a rainforest flora (Macphail *et al.* 1991). This suggests that few barriers to dispersal would have existed and the crayfish may have been far more widespread than at present. They may even have dispersed into what is now Victoria, as Bass Strait was not an open waterway until the mid Miocene. They may have been present in areas of Tasmania from which they are now absent, such as the Ben Lomond region. Environmental domain models developed by the GIS Unit, DPIWE Hobart (D. Peters, unpublished), using several parameters including relief, slope, aspect, temperature, rainfall, soil nutrients, suggest that present-day conditions in the Ben Lomond area are suitable for *O. decemdentatus, O. huonensis* and *S. catinipalmus,* at least (no other species were modelled).

In material from the Early Tertiary (~50-30 Mya) there is evidence of a change in rainforest species morphology which appears to be a response to decreasing temperature and a change in rainfall pattern (Hill *et al.* 1993), and this may have

marked the beginning of a series of range reductions for the ancestral crayfish species. A temperature decline during the Late Eocene (~35 Mya) to the Oligocene (~35-23 Mya) saw the retreat of some southern rainforest species, present in Tasmania until that time, towards the equator. In Australia there is clear evidence of increasing aridity by the Miocene, however, conditions were still wetter than today (Vickers-Rich and Rich 1993). The increase in aridity continued from the Middle Miocene to the present, with some perturbations, for instance, increased humidity during the Early Pliocene (~5-1.5 Mya). Fossil records at Alcoota in the Northern Territory, Australia, indicate a rapid change from rainforest dominated communities to communities better adapted to more arid woodland and savannahs in the Late Miocene (Vickers-Rich and Rich 1993). The increasing aridity during the Miocene may have forced the range of the ancestral crayfish to retreat to the wetter, higher regions of southern and western Tasmania. This contraction also coincided with the formation of the genera *Ombrastacoides* and *Spinastacoides*. *Spinastacoides* appears not to have been greatly influenced by further vicariant events. A step-wise expansion of range, followed by isolation, provides an adequate explanation for the phylogeny and geographical congruence of *Spinastacoides* species.

However, it seems that further range expansion occurred, and another vicariant event, possibly associated with the formation of Bass Strait, resulted in allopatric speciation of the ancestral forms of *Ombrastacoides*. If vicariant events caused these speciation events, it is highly likely that different events, or different aspects of these events, resulted in different speciation events. For example, some of the speciation events may be due to isolation during a glacial event during the Miocene, especially if ecological conditions changed after the glaciation, preventing the re-establishment of contact between some populations (maintaining barriers to dispersal). Local extinctions during vicariant events may account for the apparent non-relatedness of some groups seen in the allozyme phylogenies.

While the above scenario may explain vicariant speciation in these genera, there were other vicariant events during this period which may also have affected speciation. Carbon records in deep-sea sediments over the past 125 Myr suggest orbital forcing of the climate at regular intervals (Herbert 1997); Milankovitch cycles, well established during the Pleistocene, appear to have present for much

longer. Each of these cycles is not necessarily associated with glacial events however; changes from dry to wet were sometimes associated with monsoon-like activity instead. Around 2.6 Myr ago (Pliocene), one cycle appears coincident with the beginning of a Northern Hemisphere glacial event (Bloemendal and deMenocal 1989, Webb and Bartlein 1992), and changes in carbon isotropic records of benthic Foraminifera of mid-Miocene age are attributed to climate cooling (Woodruff and Savin 1991). While there is evidence from cores that most, if not all, cycles affected both Hemispheres at the same time, evidence does suggest that both Hemispheres were not necessarily effected to the same extent (Herbert 1997). However, Milankovitch cycles are usually too short for gradual speciation to occur in populations isolated by the event; conditions either revert back to previous conditions and the populations are in contact again, or the incipient species goes extinct because conditions have changed too much for it to cope (Dynesius and Jansson 2000). Many species appear able to survive Milankovitch oscillations; Stanley (1985) suggested that fossil records show that species' life spans vary from one to 30 million years (Myr). Webb and Bartlein (1992) also provide evidence of long-term survival (one to ten Myr) for species, and imply that most species are able to cope with changing climatic conditions by a variety of means, for example habitat tracking. They also point out that these climate changes can produce ecological changes as well, producing ecosystems with no modern analogies; that is, species cope with not only changing climatic conditions but also changes in the composition of flora and fauna associated with the species.

During the late Miocene and early Pliocene (8-6 Myr ago) significant faunal turnover has been recorded in Pakistan, North America, South America, Europe and Africa, with grazing animals becoming more dominant and replacing tropical forest and woodland adapted animals (Cerling *et al.* 1997). There is still considerable speculation as to the cause of these changes, but climate change, immigration and ecological factors have been implicated. One ecological factor implicated in this faunal turnover is the expansion of C4 grasses on a global scale beginning in the Late Miocene, due to decreases in the carbon dioxide content of the atmosphere. C4 plants are favoured by warmer temperatures and lower carbon dioxide levels.

When examining molecular phylogenetic affinities of all freshwater crayfish, the branch lengths of the Southern Hemisphere freshwater crayfish groups are longer than those seen in the Northern Hemisphere crayfish, suggesting the divergences are more ancient in the former groups (Crandall *et al.* 2000b). I suggest that this is evidence that recent vicariant events, such as the most recent Pleistocene glaciations, did not have as great an impact on speciation in Southern Hemisphere freshwater crayfish, as they did on Northern Hemisphere crayfish taxa. The evidence from the molecular studies carried out for this thesis (Chapters 2 and 3) suggests an ancient lineage for the *Ombrastacoides* and *Spinastacoides* groups; speciation and distribution patterns had, to a great extent, been established well before the last Pleistocene glacial events. Indeed, my analyses of molecular data all suggest that speciation events occurred in the Miocene rather than in the Pleistocene. Even if it is not possibly to predict which events during the Miocene were responsible for speciation in the freshwater crayfish taxa in Tasmania, clearly there were many potential factors active during this time, either of themselves or in combination, which could have triggered these events.

While the molecular data strongly suggest that Pleistocene glaciations did not instigate speciation events in these genera, we do not know whether these events had other severe effects. Extinctions of restricted species during early glacial episodes is a possibility. It is known that Pleistocene glacial events impacted on the vegetation favoured by *Ombrastacoides* and *Spinastacoides*, in regions now occupied by species of these taxa. For example, fossil evidence from Regatta Point in western Tasmania indicates the presence of species of Proteaceae in the early Pleistocene which became extinct during the earlier glacial events; overall the number of Proteaceae species in western Tasmania has not increased since the early Pleistocene, and may in fact have declined. Could this reflect the history of *Ombrastacoides* and *Spinastacoides* species, with small local extinctions during the coldest phases of glaciation? Certainly the Central Plateau region would have had local population, if not species, extinctions during the first glacial event approximately 970,000 years ago, when an ice sheet 600m deep extended across to the Tyndall Range (see Chapter 6.2). However, generalist species have less risk of their habitats disappearing totally during climate change cycles, and therefore do not need to habitat-track as much as species with more specialist requirements; they may even survive *in situ* (Dynesius

and Jansson 2000). Species subjected to large climatic changes during Milankovitch cycles should exhibit little specialisation to contemporary environments (Dynesius and Jansson 2000). These hypotheses suit *Ombrastacoides* and *Spinastacoides* species well, and I suggest that, apart from some contraction of the range of the species, the later Pleistocene glacial events had little impact on these taxa.

Phylogenetic Affinities

The phylogenetic relationships of most of the Parastacidae (excluding the Madagascan genus *Astacopsis*) have recently been analysed using sections of the 16s mtDNA genome by Crandall *et al.* (1999) and Crandall *et al.* (2000a).

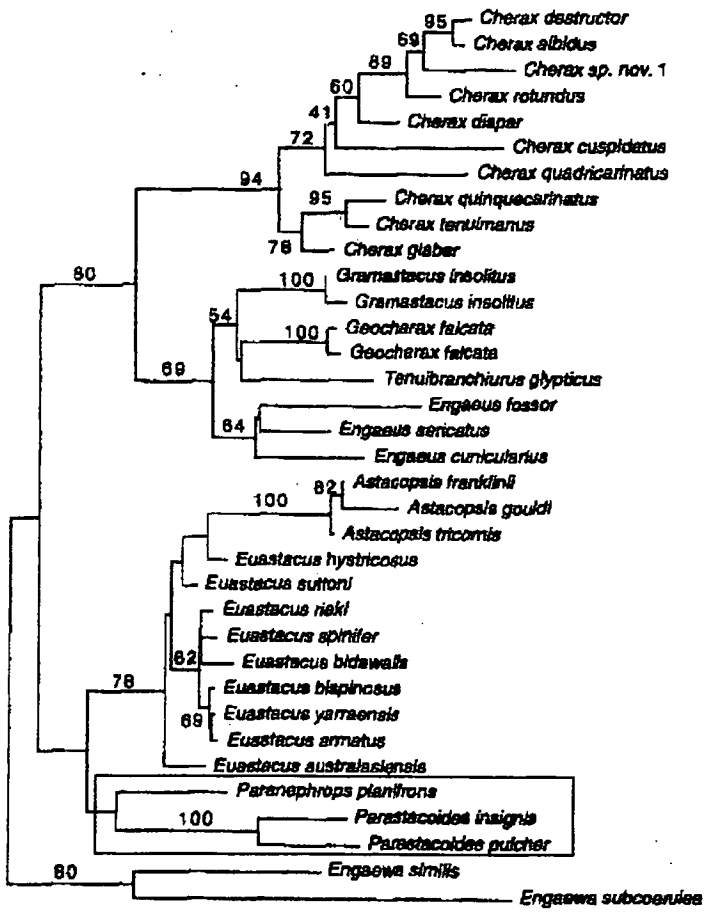


Figure 7.1. *Phylogenetic relationships among the Australian and New Zealand parastacids.* Ombrastacoides *(*Parastacoides pulcher*)* Spinastacoides *(*Parastacoides insignis*)and the New Zealand species (*Paranephrops planifrons*) are included in the red box.* Crandall *et al.* (2000a)

From the point of view of the taxa studied in this project, perhaps the most interesting observation to make from these two studies is the relationship between

the *Ombrastacoides* and *Spinastacoides* group with, firstly the New Zealand genus *Paranephrops*, and secondly with the South American genera (see Figures 7.1 and 7.2). Crandall *et al.* (1999) places *Parastacoides insignis* (*S. insignis*) and *P. pulcher* (*O. pulcher*) in a separate clade with the New Zealand *Paranephrops planifrons*. The study by which included the South American genera, as well as the Australian and New Zealand genera, showed clear support for the monophyly of the

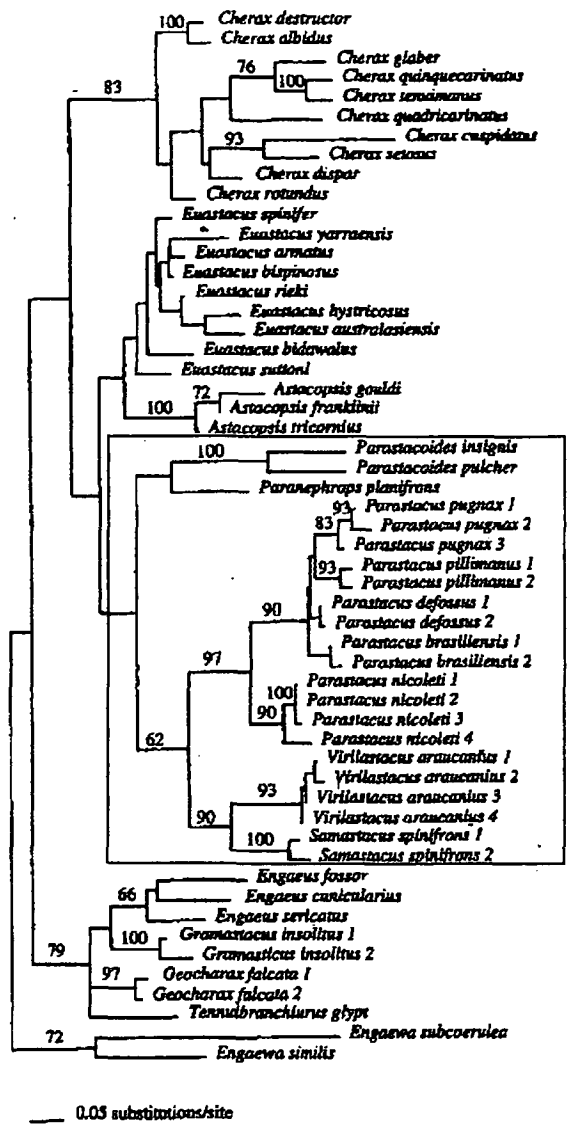


***Figure** 7.2. Phylogenetic relationships among the parastacids (excluding the Madagascan* Astacoides*).* Ombrastacoides *(*Parastacoides pulcher*)* Spinastacoides *(*Tasmanicus insignis*)and the New Zealand species (*Paranephrops planifrons*) are included with the South American species in the red box.*

South American taxa, as well as their sister group relationship with the *Paranephrops, Ombrastacoides* and *Spinastacoides* clade. These relationships allow one to suggest hypotheses regarding the timing of origin of these taxa.

The geographical distribution of these taxa, in Tasmania, New Zealand and South America, as well as the strong support given to their monophyly (Crandall *et al.* 2000a), imply that the common ancestor of these crayfish must have originated in Gondwana by the Cretaceous (~145-65 Myr ago) when these landmasses were still connected. Tasmania and New Zealand were last connected approximately 80-60 Myr ago. The Drake Passage (which separated the South American continent from Antarctica) and the South Tasman Rise (which separated Australia from Antarctica) began to appear at approximately the same time, during the Eocene (~40 Myr ago). This timing is not fully reflected in the phylogeny (Figure 7.1), that is, the separation between the South American taxa and Tasmanian-New Zealand taxa occurred earlier than the separation of the Tasmanian and New Zealand taxa. However, distance may account for the phylogenetic disparity; the origin of the ancestral taxa was either near South America, and the animals dispersed across Antarctica to Tasmania and then to New Zealand, or the taxa originated near Tasmania, with one group dispersing very early across Antarctica to South America, and another group later dispersing toward New Zealand. Riek (1972) also suggested a sister taxa relationship between *Parastacoides* and *Paranephrops*, based on morphological characters.

Burrowing habit

Horwitz and Richardson (1986) developed a classification system for the burrows of freshwater crayfish, based on the relationship of the burrow to the water table. All Astacidae are confined to streams and lakes, with burrowing activity confined to excavations in the stream beds and banks (Hobbs 1988); they only construct Type 1a or 1b (Horwitz and Richardson 1986) burrows. While some species of Cambaridae are known to excavate Type 2 burrows, the majority of species construct Type 1a or 1b burrows, and none are known to excavate Type 3 burrows (Hobbs 1988); however there is the possibility of a fossil Type 3 burrow in Texas, U.S.A. (Hasiotis *et al.* 2000). Parastacidae species are capable of constructing Type 1a, 1b, Type 2, and

occasionally Type 3 burrows. *Ombrastacoides* and *Spinastacoides* species construct Type 1a, 1b and Type 2 burrows (Chapter 6.2).

Knott (1975) suggested that the development of the Type 2 burrowing habit in *Parastacoides* (*Ombrastacoides* and *Spinastacoides*) species may have been a response to the systematic drying of lakes since deglaciation in the western region of Tasmania. However fossil evidence of crayfish burrows described by Hasiotis and Kirkland (1996) suggest that the development of Type 2 burrows had become well established in some American crayfish species by the Permian, and perhaps even as early as the Carboniferous. There is also evidence for Type 2 crayfish burrows during the Jurassic in Europe. This suggests that Type 2 burrows had developed in crayfish before the breakup of Pangaea into Laurasia and Gondwana, and there is no reason to believe that parastacid crayfish had not also developed the burrowing habit during this time. The hypothesised ancestral state for the Parastacidae genera studied by Crandall *et al.* (1999) was that of a moderate, or Type 1b (Horwitz and Richardson 1986), burrow-constructing animal. They further suggest that convergent evolution related to burrowing habits is responsible for morphological and molecular differences, and that the invasion of terrestrial habitats has taken place on several occasions among the Australian freshwater crayfish taxa. As no fossils, either body or trace, of any freshwater crayfish have been found in Tasmania it is not possible to prove that they had also developed their burrowing habit before Pleistocene glacial events. However, studies on the composition of the "pholeteros" (Lake and Newcombe 1975, Richardson and Swain 1978, Horwitz 1989, Horwitz and Knott 1991), the faunal assemblage associated with crayfish burrows (Lake 1977), suggest a long association. Early studies of the pholeteros suggested that only a limited range of fauna was present (for example Lake and Newcombe (1975) found only crustaceans), but later studies have greatly expanded the range of fauna found. Horwitz (1991) found 27 taxa associated with crayfish burrows, with the burrows in sedgeland having more taxa than those in rainforest. In his 1989 study, Horwitz argued that some taxa were exclusive dwellers in the burrow habitat in alpine conditions in southwest Tasmania, particularly in the larval stage. He suggested that not only drought conditions, but also cold temperatures may have facilitated the establishment of this fauna. Whatever the circumstances leading to the association in the past, the fact that some of these taxa are only found associated with crayfish

burrows, and not with other forms of free-standing water in the immediate vicinity, argues for the association to be a long-term and sustained one. If it were recent, one would expect to find the taxa in nearby waters.

That both *Ombrastacoides* and *Spinastacoides* species are strong burrowers also argues for this being an ancient habit. The molecular evidence suggests a split between the two genera during the Miocene, and I suggest that either the ancestral taxon was already a strong burrower or that the increasing aridity created the environment for the evolution of the Type 2 burrowing habit in both these genera. The fact that *Paranephrops* is usually associated with stream habitats, and not burrows remote from standing water, may suggest that the ancestral taxon to *Paranephrops* and *Ombrastacoides/Spinastacoides* was not a strong burrower, perhaps constructing Type 1a burrows. This suggests that the increasing aridity during the Miocene may have played a role in the development of Type 2 burrow construction in the Tasmanian taxa.

Conservation issues

The revised taxonomy of *Parastacoides* presented here raises conservation concerns not apparent with the previous review. *Parastacoides tasmanicus tasmanicus* (Sumner 1978) was well protected as the majority of its distribution lay within the boundaries of the Western Tasmania World Heritage Area (Hansen and Richardson 1999c). However, four of the newly recognised species, *O. denisoni, O. dissitus, O. parvicaudatus* and *O. professorum*, have distributions which lie entirely outside of the World Heritage Area, and all four of these species have restricted ranges. Table 8.1 provides a summary of the criteria used in the Tasmanian Threatened Species Protection Act 1995. Using these criteria, five species raise conservation concerns, as they may meet the criteria for endangered, vulnerable or rare. *Ombrastacoides parvicaudatus* may well be already extinct, as all known collection localities are now under water in the hydro-electric storage created by the Lake Burbury dam. The same fate may have befallen *O. pulcher,* as the two largest collection localities are now beneath Lake Pedder (artificially enlarged due to a hydro-electric dam), with only a few isolated specimens collected outside this area. Of most current concern is *O. denisoni.* It is known from only one collection locality, and has a known distribution range of only about one square kilometre (see Chapter 6.1); the area

surrounding its range has been partly cleared, and is in an area designated for future forestry clearing. Two further species, *O. dissitus* and *O. professorum*, may warrant listing, due to their very restricted ranges (both less than 20 square kilometres (see Chapter 6.2)).

Table 7.1. *A summary of criteria used in the Tasmanian Threatened Species Protection Act 1995 (after Richardson et al. 1999), with suggested classification of some species.*

Status	Population reduction	Extent of Occurrence	Population Size	Species
Endangered	>50% in past 10 years >50% projected in next 10 years	<5000 km^2 occurrence <500 km^2 occupancy **+ any 2 of** i. fragmented (<5 locations) ii. declining iii. extreme fluctuations	<250 or <25000 **+ either** *i.*20% decline in 5 years *ii.* declining **+ either** fragmented (all pop. < 1000), **or** all in 1 pop.	*O. denisoni* ? *O. parvicaudatus* ? *O. pulcher* ?
Vulnerable	>20% in past 10 years >20% projected in next 10 years	<20, 000 km^2 occurrence <2000 km^2 occupancy **+ any 2 of** i. fragmented (<5 locations) ii. declining iii. extreme fluctuations	<250 or <25000 **+ either** *i.* 10% decline in 10 years *ii.* declining **+ either** fragmented (all pop. < 1000), **or** all in 1 pop.	*O. denisoni* ? *O. parvicaudatus* ? *O. pulcher* ? *O. professorum*?
Rare	A. limited range **+ threatening processes:** *r1*: extent <100 x 100 km *r2*: found in <20 10x10 km grids *r3*: pop. always small & localised	B. Stochastic risk, pop. naturally small: Extent <2000 km^2 **or** occupancy <50 ha **or** pop. <1000 **or** no pop. > 1000 **or** most animals in <10 pops		*O. dissitus* ? *O. professorum*?

As the main factors limiting the distribution of *Ombrastacoides* and *Spinastacoides* species appear to be the availability of sufficient water (more than 1000 mm per year coupled with an evaporation rate less than the rainfall (Chapter 6.2)), the climatic warming associated with the increase in atmospheric greenhouse gases may have some impact on the continued survival of some of the species. Increasing temperature on its own is unlikely to have a serious impact, unless it is accompanied by a decrease in precipitation, or an increase in the evaporation rate. Opinion on the

exact impacts of global warming associated with greenhouse gases varies. It is generally agreed that a rise of between 1.5 - 4.5°C (2.5°C the most likely) is to be expected by the middle of this century (Singh 1997). However, many of the models also predict a change in precipitation patterns (McGuffie *et al.* 1999). The general consensus appears to be that precipitation patterns will alter, with, for example a decline in light and medium rainfall events, but an increase in the heaviest events, particularly in winter (McGuffie *et al.* 1999 and Osborn *et al.* 2000). Extreme events, such as floods and droughts are also expected to increase (Lal and Bhaskaran 1993, McGuffie *et al.* 1999 and Osborn *et al.* 2000). Overall, the number of days with cloud is expected to increase, as is the humidity (Lal and Bhaskaran 1993, McGuffie *et al.* 1999 and Osborn *et al.* 2000). No modelling has been attempted at the local scale for Tasmania (Prof. W. Budd, CRC Antarctic and Southern Ocean Environment, University of Tasmania, pers. comm.); modelling of climate change for the Australian region includes Tasmania as a single 5°x5° grid square (Karl 1998). However, research by the CSIRO's Climate Change Research Program CSIRO (http://www.dar.csiro.au/res/cm/jpegForWeb1999/tas.htm 26/03/2001) suggests that there has been a steady decrease in annual rainfall in Tasmania (see Figure 8.3), and this trend is expected to continue. Although winter rainfall appears to be increasing, the overall trend is for a decrease in precipitation. The effects of this will be most apparent in summer, with an increase in the evaporation rate due to increased temperature (Prof. W. Budd, CRC Antarctic and Southern Ocean Environment, University of Tasmania, pers. comm.). This suggests that some of the species would experience difficulty with the expected changes associated with global warming. *Ombrastacoides denisoni* and *O. dissitus* are perhaps the most vulnerable, as they have restricted ranges at the eastern boundary of the genus range.

Species with larger distributions could expect some contraction of their range, particularly *O. leptomerus* in the north. This would be due not only to climate change but also to increased land clearing and forestry in the north. A possible solution to these problems may be either leaving or the creating of "corridors" of suitable habitat to allow animals to retreat to and recolonise more suitable environments in response to environmental changes. Although we know the present rate of dispersal is slow, we do not know at what rate dispersal can occur when the animals are stressed; they

may be able to respond to climate change and relocate at a pace that will allow them to keep pace with climate change.

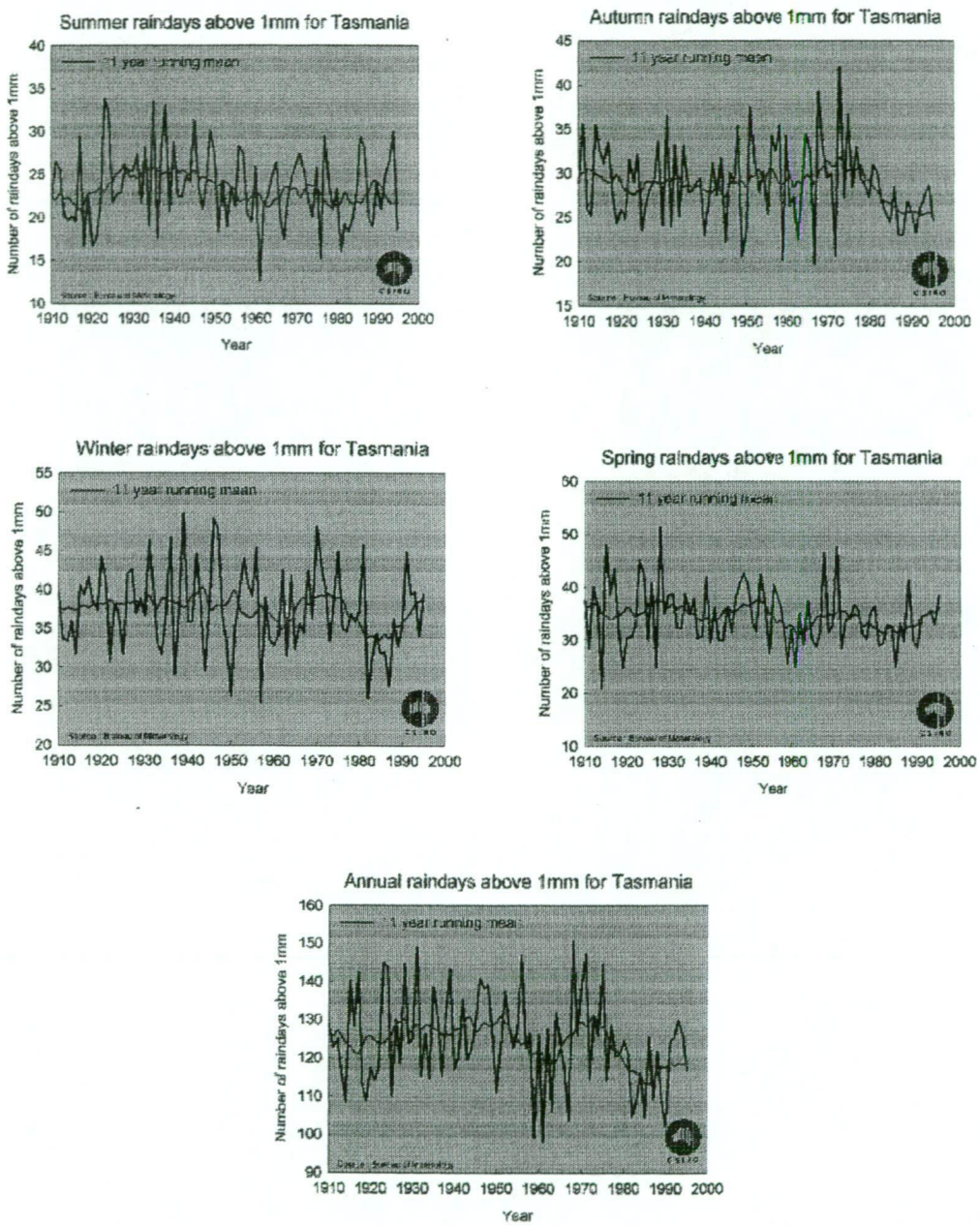


Figure 7.3. *Rainfall events over 1mm in Tasmania from the years 1900 to 2000, showing seasonal as well as annual averages (from CSIRO http://www.dar.csiro.au/res/cm/jpegForWeb1999/tas.htm 26/03/2001).*

Further Research

At least three avenues for further research relating to speciation arise from the present study: 1) analysis to determine whether chromosomal rearrangements have

led to speciation events, 2) determining whether timing of mating has provided isolating mechanism in some instances, and 3) further investigation of the geographical pattern of speciation based on the methods developed by Barraclough and Vogler (2000).

Karyological Studies

The incidence of sympatry between species that are distinguished by very small phenotypic differences, but which differ strongly at the molecular level, raises the problem of how to explain the persistence of the separate species over time. Translocations, inversions or changes in the number of chromosomes can all lead to rapid speciation (Avise 1994) without apparent morphological or ecological changes. Chromosome analysis may prove difficult with these taxa however. As mentioned (Chapter 3.2) freshwater crayfish have a very large number of small chromosomes (up to 365 for some Australian species). Some types of analyses, for example fluorescence activated cell sorting (FACS) are more difficult in taxa that have a large number of similar-sized chromosomes (Sessions 1996). However, the very large numbers involved at the chromosome level does suggest that the possibility of chromosome changes creating speciation in these taxa is highly likely.

Reproductive Isolating Mechanisms

Behaviour is another isolating mechanism which has the ability to stabilise closely related sympatric species (Schreiber *et al.* 2000). One parapatric pair (*O. decemdentatus* and *O. huonensis*) appear to have different spawning times, with *O. decemdentatus* spawning two or more months earlier than *O. huonensis* (A. Richardson, University of Tasmania, pers. comm.). Usually the gonads of reproducing females and males exhibit synchronous cyclic development (Hamr and Richardson 1994), so a delay of onset for one species may be enough to create a prezygotic mating barrier. Regardless of the reproductive isolating mechanism involved, prezygotic mating behaviour or postzygotic cytogenetic incompatibility, or other yet to be established factors, the large genetic interspecies distance indicates a very ancient speciation event maintained for a considerable period of time..

Historical Range Analysis

Barraclough and Vogler (2000) argue that, if speciation is recent and allopatric in origin, then the distribution of closely related sister taxa should show little or no overlap. However, if speciation is ancient and allopatric, or recent and sympatric in origin, then ranges may overlap. Dispersal rates have an obvious impact on the degree of overlap, related to the age of species in these cases. However, if species have very similar habitat requirements then allopatric distributions may be maintained through a mechanism of competitive exclusion (Letcher *et al.* 1994). If we follow the arguments of Barraclough and Vogler (2000), the distributions of the species in this study could suggest evidence of: 1) recent allopatric speciation, as there is little overlap in distribution; 2) ancient allopatric speciation with range shifts due to historical factors; or 3) ancient sympatric speciation with range shifts due to historical factors. As the molecular data show that the speciation events were ancient, the information on current geographical ranges alone is unlikely to be sufficient to allow a prediction of the mode of speciation. More work in this area may elucidate modes of speciation in some of the species discussed in this thesis.

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Appendix A – Molecular Protocols for 16S gene

DNA extraction methods

1. CTAB Extraction Protocol

- use a sterile scalpel blade, open pack and use pack as a sterile cutting surface
- remove approximately 100mg of tissue
- rinse tissue in dH_2O (milliQ)
- discard dH_2O, rinse beaker with dH_2O, and replace dH_2O after every specimen
- place tissue in sterile microcentrifuge tube containing 200 µl CTAB (Hexadecyltrimethylammonium bromide) buffer
- grind tissue using a plastic pestle to form a homogenous slurry
- add a further 400µl of CTAB buffer, and regrind
- add 5µl of 20mg/ml proteinase K
- vortex and incubated for 1 hour at 65°C, vortex occasionally throughout this period
- in fume hood, add 600µl chloroform iso-amyl alcohol (24:1 mix)
- centrifuge for 20 minutes at 13,000rpm
- place 600µl of phenol/chloroform-isoamyl alcohol (25:24:1) in a new tube, using a pre-wet filter-tip
- remove upper aqueous layer, using a blue tip
- add to tube with phenol/chloroform iso-amyl.
- mix for 1 minute, rest for 30 seconds, mix briefly
- centrifuged at 13,000rpm for 10 minutes (where necessary repeat the previous step until a clear upper aqueous layer is formed)
- remove the aqueous upper layer, place in a new tube with 600µl of chloroform iso-amyl alcohol (24:1) to remove any trace of phenol, mix well
- centrifuge at 13000rpm for 30 seconds
- transfer the aqueous layer to a new tube with 1.5 volumes (700 - 900µl) of cold (-20°C) iso-propanol
- invert tube gently approximately 10 times till a pellet of DNA formed
- allow DNA to precipitate overnight at - 20°C
- centrifuge the DNA for 20 minutes at 13,000 rpm
- remove the supernatant
- add 200 - 500µl of 70% cold ETOH
- invert the tube gently
- centrifuge for 10 minutes (13,000 rpm) in a cold room
- dry the DNA pellet under vacuum for approximately 5-15 minutes
- resuspended in 50 - 100ml of dH_2O
- rehydrate overnight at 4°C.
- mix (flick mixS) and transfer to freezer

2. Chelex/Instagene DNA Extraction

- remove 25mg tissue - macerate on foil packaging of a sterile scalpel blade – place in 1.5 ml microcentrifuge tube
- place instagene (as per BIORAD) matrix on a magnetic stirrer
- add 200µl instagene matrix and 2µl of 20mg/ml proteinase K to tissue
- vortex and incubate at 56°C for 2 hours (vortex every half hour)
- vortex for 10 seconds and place into a boiling water bath for 10 minutes
- vortex for 10 seconds every 3 minutes
- vortex for 30 seconds and place on ice for 1 minute
- centrifuge at 13,000 rpm for 2 minutes
- store DNA in freezer

3. QIAGEN DNeasy Tissue Kit

- remove 25mg tissue – cut into small pieces and place in 1.5ml microcentrifuge tube
- add 180µl Buffer ATL (supplied in kit)
- add 20µl Proteinase K, vortex and incubate at 55°C until tissue lysed (approximately 1 hour)
- vortex, add 200µl Buffer AL (supplied in kit)
- vortex and incubate at 70°C for 10 minutes
- add 200µl ethanol (96%), vortex
- pipette resultant mixture into DNeasy mini column in a 2ml collection tube
- centrifuge at >6000 rpm for 1 minute
- place DNeasy mini column in new 2ml collection tube – discard flow-through
- add 500µl Buffer AW1 (supplied in kit)
- centrifuge for 1 minute at >6000 rpm
- place DNeasy mini column in a 2ml collection tube – discard flow-through and old collection tube
- add 500µl Buffer AW2 (supplied in kit)
- centrifuge for 1 minute at >6000 rpm
- place DNeasy mini column in 1.5ml microcentrifuge tube – discard flow-through and collection tube
- add 200µl distilled water directly onto DNeasy membrane (protocol differs from manufacturer)
- incubate at room temperature for 1 minute
- centrifuge for 1 minute at >6000 rpm
- store in DNA freezer

4. CTAB Extraction Protocol (modified using PVP)

- use a sterile scalpel blade, open pack and use pack as a sterile cutting surface
- remove approximately 100mg of tissue
- rinse tissue in dH_2O (milli Q)
- discard dH_2O, rinse beaker with dH_2O, and replace dH_2O after every specimen
- place tissue in sterile microcentrifuge tube containing 200 µl CTAB (Hexadecyltrimethylammonium bromide) buffer
- add 2% (0.1g) PVP (polyvinylpyrrolidone)
- grind tissue using a plastic pestle to form a homogenous slurry
- add a further 400µl of CTAB buffer, and regrind
- add 5µl of 20mg/ml proteinase K
- vortex and incubated for 1 hour at 65°C, vortex occasionally throughout this period
- in fume hood, add 600µl chloroform iso-amyl alcohol (24:1 mix)
- centrifuge for 20 minutes at 13,000rpm
- place 600µl of phenol/chloroform-isoamyl alcohol (25:24:1) in a new tube, using a pre-wet filter-tip
- remove upper aqueous layer, using a blue tip
- add to tube with phenol/chloroform iso-amyl.
- mix for 1 minute, rest for 30 seconds, mix briefly
- centrifuged at 13,000rpm for 10 minutes (where necessary repeat the previous step until a clear upper aqueous layer is formed)
- remove the aqueous upper layer, place in a new tube with 600µl of chloroform iso-amyl alcohol (24:1) to remove any trace of phenol, mix well
- centrifuge at 13000rpm for 30 seconds
- transfer the aqueous layer to a new tube with 1.5 volumes (700 - 900µl) of cold (-20°C) iso-propanol
- invert tube gently approximately 10 times till a pellet of DNA formed
- allow DNA to precipitate overnight at - 20°C
- centrifuge the DNA for 20 minutes at 13,000 rpm
- remove the supernatant
- add 200 - 500µl of 70% cold ETOH
- invert the tube gently
- centrifuge for 10 minutes (13,000 rpm) in a cold room
- dry the pellet of DNA under vacuum for approximately 30 minutes
- resuspended in 50 - 100ml of dH_2O
- rehydrate overnight at 4°C.

mix (flick mix) and transfer to freezer

5. CTAB Extraction Protocol (modified using PEG)

- use DNA extracted previously
- bring volume up to 200µl with dH_2O
- add 80µl polyethanolglycol (30% PEG + 1.4M NaCl) (=40% volume)
- mix
- precipitate at 0-4°C overnight
- centrifuged at 13,000rpm for 20 minutes (at 4°C)
- remove supernatant
- resuspend pellet in 100µl dH_2O for minimum 1 hour
- add 10µl sodium acetate (10% NaAc)
- add 2 volumes (200µl) 95% cold ETOH
- allow to stand for minimum 10 minutes at -20°C
- centrifuged at 13,000rpm for 20 minutes (at 4°C)
- remove supernatant
- add 200 - 500µl of cold 70% ETOH
- invert the tube gently
- centrifuge for 10 minutes (13,000 rpm) in a cold room or refrigerated centrifuge
- dry the pellet of DNA under vacuum for approximately 5-15 minutes
- resuspended in 30-50µl of dH_2O
- rehydrate overnight at 4°C.

mix (flick mix) and transfer to freezer

PCR Reaction Mix

1. Standard PCR Reaction mix

If using DNA extract for the first time : dilute 1:10 with dH_2O in labelled 0.5ml tubes.

Material: Quantity (for 1 tube)

Sterile dH_2O: 31.3µl, 10x buffer: 5µl, MgCl (25 mM stock)$_2$: 4µl, Dntps (25mM stock): 0.5µl, Primer 1 (10µM): 1µl, Primer 2 (10µM): 1µl, Taq (5-10 units per µl):0.2µl , BSA (10 mg/ml): 5µl

- prepare and number 0.5ml tubes
- mix buffer PCR mix for all samples (+1) in 1.5ml tube
- mix and spin down for 1 second
- add 48µl of the buffer PCR mix solution to the numbered tubes
- Add 2µl DNA into each numbered tube (total = 50µl)
- Add 2µl dH_20 to make the 50µl negative control

2. Crandall and Fitzpatrick (1996) PCR buffer mix

If using DNA extract for the first time : dilute 1:10 with dH_2O in labelled 0.5ml tubes.

Material: Quantity (for 1 tube)

Sterile dH_2O: 32.3µl, 10x buffer: 5µl, MgCl (25 mM stock)$_2$: 4µl, Dntps (25mM stock): 0.5µl, Primer 1 (10µM): 2.5µl, Primer 2 (10µM): 2.5µl, Taq (5-10 units per µl):0.2µl

- prepare and number 0.5ml tubes
- mix buffer PCR mix for all samples (+1) in 1.5ml tube
- mix and spin down for 1 second
- add 48µl of the buffer PCR mix solution to the numbered tubes
- Add 2µl DNA into each numbered tube (total = 50µl)
- Add 2µl dH_20 to make the 50µl negative control

3. Modified PCR buffer mix

If using DNA extract for the first time : dilute 1:10 with dH_2O in labelled 0.5ml tubes.

Material: Quantity (for 1 tube)

Sterile dH_2O: 30.3µl, 10x buffer: 5µl, MgCl (25 mM stock)$_2$: 5µl, Dntps (25mM stock): 0.5µl, Primer 1 (10µM): 1µl, Primer 2 (10µM): 1µl, Taq (5-10 units per µl):0.2µl , BSA (10 mg/ml): 5µl

- prepare and number 0.5ml tubes
- mix buffer PCR mix for all samples (+1) in 1.5ml tube
- mix and spin down for 1 second
- add 48µl of the buffer PCR mix solution to the numbered tubes
- Add 2µl DNA into each numbered tube (total = 50µl)
- Add 2µl dH_20 to make the 50µl negative control

PCR Temperature Profile

1. Standard

initial denaturing at 94°C for 4 min (1 cycle), (94°C – 30 secs, 55°C – 1 min, 72°C – 1 min 30 secs) 35 cycles, 72°C – 5 mins, 4°C.

2. Crandall and Fitzpatrick 1996

initial denaturing at 96°C for 3 min (1 cycle), 95°C – 20 secs, 41°C – 30 secs, 72°C – 2 mins (45 cycles), 72°C – 5 mins (1 cycle), 4°C.

3. Standard Modified (1)

initial denaturing at 94°C for 5 min (1 cycle), (94°C – 30 secs, 55°C – 30 secs, 72°C – 1 min, 94°C - 30 secs, 48°C – 30 secs, 72°C – 1 min) 35 cycles, 72°C – 5 mins, 4°C.

4. Standard Modified (2)

initial denaturing at 94°C for 5 *min* (1 cycle), (94°C – 30 secs, 55°C – 30 secs, 72°C – 1 min) 10 cycles, (94°C - 30 secs, 48°C – 30 secs, 72°C – 1 min) 25 cycles, 72°C – 5 mins, 4°C.

5. Standard Modified (3)

initial denaturing at 94°C for 5 min (1 cycle), (94°C – 30 secs, 46°C – 30 secs, 72°C – 1 min) 5 cycles, (94°C - 30 secs, 50°C – 30 secs, 72°C – 1 min) 30 cycles, 72°C – 5 mins, 4°C.

6. Standard Modified (4)

initial denaturing at 94°C for 5 min (1 cycle), (94°C – 30 secs, 48°C – 30 secs, 72°C – 1 min) 5 cycles, (94°C - 30 secs, 52°C – 30 secs, 72°C – 1 min) 30 cycles, 72°C – 5 mins, 4°C.

Electrophoresis

- agarose – recycled gel or (1% mix for large electrophoresis tank and large fragments eg 0.6g for 60ml) (1.5% for small fragments)
- microwave in short bursts until totally dissolved
- cool till safe to handle
- set up appropriate tank and pour in agrose gel mix
- slot in combs
- when gel solidifies (approximately 10 – 15 minutes) remove combs and/or end blocks
- add 1xTBE buffer to cover gel
- pipette 2µl of loading buffer (blue dye) onto parafilm for each DNA sample
- add 5µl of DNA sample to blue dye and mix well
- adjust pipette to 7µl, pick up dye+ DNA and inject into gel well
- add 5µl size standard to the last well
- connect power leads
- run at 86 volts (for small tank) or 130 volts (for large tank) for approximately 30 minutes

Gel Examination

- add 5 (6)µl EtBr to buffer.
- place on shaker tray for 20 minutes (1/3rd speed)
- Pour off EtBr buffer
- rinse gel in water
- add water to tray and place on shaker for 2-5 minutes to destain
- check gel with U/V light (wearing face shield)
- print image

DNA Purification

1. Concert Rapid PCR Purification System (Life Technologies Inc.)

- trasnsfer PCR reaction into 1.5ml tubes
- transfer 400µl H1 solution to tubes
- mix
- transfer solution to columns (inserted into collection tubes)
- centrifuge for one min
- discard flowthrough
- insert columns into new collection tubes
- add 700µl H2 solution
- centrifuge at 16 000 rpm for one min
- discard flowthrough
- re-centrifuge for one min
- insert columns into new 1.5ml tubes
- add 30µl heated (65°C) dH_2O
- incubate at room temperature for one min
- centrifuge for two mins
- freeze

2. Qiagen – Gel extraction PCR purification

- cast new 1% agrose gel
- add EtBr to agrose
- add 4-5µl dye to 45µl of purified product
- run for 30 mins at 80 volts
- weigh 1.5ml centrifuge tubes
- visualise gel under ultra-violet light (shield bands not being cut)
- cut out band – insert gel into 1.5 ml tube – wrap tube in foil to avoid ultra-violet light
- rewiegh tubes
- add Qiagen Buffer (3-1 volumes)
- melt gel in 50°C water bath (10 minutes or til dissolved)
- add 1 gel volume Isopropanol – finger mix
- add whole volume of tube to spin column
- centrifuge at 14,000 rpm for 1 minute
- discard flow-through
- add 0.5ml QG Buffer – centrifuge again
- discard flow-through
- add 750µl Buffer PE (an ethanol wash)
- centrifuge at 14,000 rpm for 1 minute
- discard flow-through

- re-spin to remove residue
- insert columns into 1.5ml centrifuge tube
- add 30µl dH_2O directly on filter
- let stand for 1 minute
- centrifuge at 14,000 rpm for 1 minute
- store DNA product in freezer

3. Amersham GFX Gel Band Purification Kit - – Gel extraction PCR purification

- cast new 1% agrose gel
- add EtBr to agrose
- add 4-5µl dye to 45µl of purified product
- run for 30 mins at 80 volts
- weigh 1.5ml centrifuge tubes
- visualise gel under ultra-violet light (shield bands not being cut)
- cut out band – insert gel into 1.5 ml tube – wrap tube in foil to avoid ultra-violet light
- rewigh tubes
- add 10µl per 10mg capture buffer
- vortex
- incubate at 60°C till dissolved (5-15 mins)
- place GFX column in collection tube for each purification
- centrifuge briefly when agrose melted
- transfer sample to GFX column
- incubate at room temperature for one min.
- centrifuge at 16 000 rpm for 30 secs
- discard flowthrough
- place GFX column back in collection tube
- add 500µl Wash Buffer
- centrifuge for 30 secs
- discard collection tube with flowthrough
- transfer GFX column to 1.5ml tube
- apply 30µl dH_2Odirectly onto filter
- incubate at room temperature for one min
- centrifuge for one minute
- freeze

Quantifying DNA Product

Biorad Versaflour Fluorometer Systems (Hercules, California)

DNA Assay

- prepare Flouro Buffer Dye solution
  45ml milliQ
  5ml 10xTNE buffer pH7.4 (0.2mM NaCl, 10mM Tris-Cl, 1mM EDTA)
  5µl dye (1.6nM) (H33258 binds specifically to DNA and fluoresces at set wavelength = 460nm)
- rinse cuvette with milliQ (de-ionised water)
- add 2mL Assay solution to cuvette
- set machine to 0
- set range to 0
- add 2µl calf thymus DNA (calf thymus DNA used as reference = 100mg/µl) – mix with pipette
- set range to 100 (reference point)
- discard solution and rinse cuvette in milliQ
- add 2mL assay solution – should read 0 ± 5 or rezero
- add 2µl DNA – mix

Sequencing Protocol

DNA Purification

1 ABI Prism Dye Terminator Cycle Sequencing ready reaction kit

- dilute primers to 5 µM
- aim for 30-40 nanograms in general (use standard PCR 0.2 ml tubes)
- total volume 10µl
  - 4µl BIGDye Terminator
  - 0.65µl primer (5 µM)
  - DNA varies (30-40 ng)
  - dH2O varies (to 10 µl)
- add in order dH_2O, primer, DNA, Dye Terminator (mix with pipette at end)
- insert into PCR machine
- DYETERMINATOR programme (initial denaturing at 96°C for 5 min (1 cycle), (96°C – 30 secs, 50°C – 15 secs, 60°C – 4 mins) 25 cycles, 4°C)

2. Amersham DYEnamic ET sequencing kit

- dilute primers to 5 µM
- aim for 30-40 nanograms in general (use standard PCR 0.2 ml tubes)
- total volume 10µl
    - 4µl BIGDye Terminator
    - 0.65µl primer (5 µM)
    - DNA varies (30-40 ng)
    - dH2O varies (to 10 µl)
- add in order dH_2O, primer, DNA, Dye Terminator (mix with pipette at end)
- insert into PCR machine
- ET TERMIN programme (initial denaturing at 96°C for 20 secs (1 cycle), (50°C – 15 secs, 60°C – 1 min) 25 cycles, 4°C)

Precipitation

1. Standard

- transfer all sequencing mix to 1.5ml tubes
- add 30µl (95%) ETOH
- add 1µl 3M NaAcetate (pH4.6)
- vortex – leave for 10 minutes on ice – vortex
- centrifuge for 25 mins at 14000rpm
- remove liquid (pellet may or may not be visible)
- add 150µl (70%0 ETOH (washes pellet) (run ETOH down opposite side to pellet to avoid dislodging)
- remove liquid immediately
- insert open tubes into vacuum dryer – temp 35-40°C
- leave 3-5 mins
- remove, check for moisture, close lid, freeze until sent for sequencing

2. Amersham DYEnamic ET

- transfer all sequencing mix to 1.5ml tubes
- add 40µl (95%) ETOH
- add 1µl (3M NaAcetate +EDTA) (from kit)
- vortex – leave for 15-20 minutes on ice – vortex
- centrifuge for 15 mins at 14000rpm
- remove liquid (pellet may or may not be visible)
- add 250µl (70%0 ETOH (washes pellet) (run ETOH down opposite side to pellet to avoid dislodging)
- remove liquid immediately
- insert open tubes into vacuum dryer – temp 35-40°C
- leave 3-5 mins
- remove, check for moisture, close lid, freeze till sent for sequencing

Appendix B has been removed for copyright or proprietary reasons.

Hansen, B., Richardson, A. M. M., 1998. Conservation implications arising from a systematic review of the Tasmanian freshwater crayfish genus Parastacoides (Decapoda: Parastacidae), Crustacean issues, 12 799-805

Appendix C has been removed for copyright or proprietary reasons.

Hansen, B., Richardson, A. M .M., 1999. A preliminary inquiry into the biogeography of the Tasmanian endemic freshwater crayfish genus Parastacoides Clark (Decapoda: Parastacidae), Freshwater crayfish 12(1), 854-861

Appendix D has been removed for copyright or proprietary reasons.

Richardson, A. M. M., Doran, N. E., Hansen, B., 1999. The conservation status of Tasmanian freshwater crayfish, Freshwater crayfish 12(1), 863-877

Appendix E has been removed for copyright or proprietary reasons.

Hansen, B. and Richardson, A. M. M. 1999. Interpreting the geographic range, habitat and evolution of the Tasmanian freshwater crayfish genus Parastacoides from a museum collection. pp 210-218 in: The Other 99% - The conservation and biodiversity of invertebrates, edited by W. Ponder and D. Lunney. Transactions of the Royal Zoological Society of New South Wales, Mossman
ISBN 0 9586085 12

Appendix F – Taxa determination

Determination of the genera *Ombrastacoides* and *Spinastacoides*:

1. Evidence from allozyme electrophoresis (Chapter 2) suggests the presence of two genera. Both allozyme studies have populations with levels of genetic divergence typical of those which characterise different genera (Nei D > 1.00; Ayala 1982, Thorpe 1982):

- A3a (*insignis*)
- A3b (*insignis*)
- A2a (*inermis*)
- A1 (*inermis*)
- B2 (*inermis*)
- B3 (*inermis*)

These populations are composed of taxa which have a terminal spine (spiny-tailed) on the uropod exopod. One population of inermis in Study A, and two populations of inermis in Study B, did not show this degree of genetic divergence from non spiny-tailed populations. However, they do still show quite large levels of genetic divergence from other populations (0.42 - 0.72).

2. Mitochondrial DNA evidence suggests large genetic divergences (Chapter 3).

- The COI gene segment (Chapter 3.1) suggests a division between the spiny-tailed (Ptis and Ptin taxa) and the non spiny-tailed taxa (Ptt). Pairwise distances, using the Kimura 2-parameter algorithm, between the two groups range from 20 - 25%.
- The 16S gene segment (Chapter 3.2) suggests a division between the spiny-tailed and the non spiny-tailed species (Figure 3.2.1). There is also a suggestion of a division including two non spiny-tailed species (WCT and LDRT). This division is not apparent in the allozyme data. Pairwise distances, using the Kimura 2-parameter algorithm, between the spiny-tailed and non spiny-tailed groups range from 13 - 19%.
- The 16S gene segment suggests that saturation levels between transitions and transversion in the spiny-tailed and non spiny-tailed groups are behaving differently (Chapter 3.2 pp 3.28 - 3.32.

3. The combined allozyme and mitochondrial DNA data (Chapter 3.2 pp 3.32 - 3.34) supports a division between spiny-tailed and non spiny-tailed taxa.

4. The morphometric analyses (canonical discriminant function analysis and MDS) support a division between the spiny-tailed and non spiny-tailed taxa based on both meristic characters and overall shape (determined from linear measurements).

5. The spiny-tailed species show a clear diagnostic character separating them from the other taxa (the presence of a terminal spine on the uropod exopod).

6. The distribution of the two groups differs. The spiny-tailed taxa are found primarily in the south of the state, while the non spiny-tailed taxa are found primarily in the west and central south (Figure 7.1.3). Where there is overlap and populations of the two groups are found in close proximity, habitat

Appendix G – Taxa nomenclature

Codes and names used for taxa within the thesis.

Species name	Code	Previous name	Allozyme code	COI code
Ombrastacoides				
O. asperrimanus	RCT	*tasmanicus*	B8b, B9	
O. brevirostris	WCT	*tasmanicus*	B7b, B8a	
O. decemdentatus	NT	*tasmanicus*	B6	Ptt2
O. denisoni	LDRT	*tasmanicus*	B5	
O. dissitus	SET	*tasmanicus*	B1	
O. huonensis	SPTA	*tasmanicus*	A2b, A2c, B4b	Ptt1
O. ingressus	VPT	*tasmanicus*	A5, B10	
O. leptomerus	LT	*tasmanicus*	A6, A7, B11a, B11b, B13a, B13b, B14, B15	
O. parvicaudatus	LMT	*tasmanicus*	B12	
O. professorum	ACT	*tasmanicus*		
O. pulcher	SPTB	*tasmanicus*		
Spinastacoides				
S. catinipalmus	WCI	*inermis*	A4, B7a	
S. inermis	IS	*inermis*	A1, A2a, B2, B4a	Ptin1
S. insignis	I	*insignis*	A3a, A3b, B3	Ptis1, Ptis2